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1887 ANNOUNCEMENTS

Doxorubicin-Vincristine Therapy for Wilms' Tumor: A Pilot Study[1,2]

Bruce Camitta,* Larry Kun, Marvin Glicklich, Herbert Oechler, Stuart Adair, and Donald Pinkel[3]

Doxorubicin plus vincristine chemotherapy was given to 31 children following nephrectomy for Wilms' tumor. Radiation therapy was used as indicated. Disease-free survival by stage is: eight of nine patients (stage I), eight of nine (stage II), nine of ten (stage III), and two of three (stage IV). Median follow-up of survivors is 28 months (range, 2–67); for all but four patients, follow-up is > 12 months. Two of the three stage I–III failures occurred in children with unfavorable histologies; the third failure was due to fatal anthracycline cardiomyopathy. Lowering the maximal cumulative doxorubicin dose from 450 to 240 mg/m^2 did not increase failures. Doxorubicin-vincristine appears to be effective chemotherapy for Wilms' tumor. [Cancer Treat Rep 66:1791–1794, 1982]

Wilms' tumor is the most common renal tumor of childhood. Although local disease can be eliminated with surgery and irradiation, 50% of children develop metastases (primarily pulmonary). The occurrence of metastases despite control of the primary tumor suggests that microscopic metastatic deposits are present at the time of diagnosis. Only one-half of the children developing macroscopic pulmonary disease during therapy remain free of disease despite additional treatment (1,2). Nonpulmonary metastases have even worse prognostic import (2). Therefore, the goal of adjuvant chemotherapy in Wilms' tumors is to eliminate metastases before they become clinically apparent.

Early trials of chemotherapy for Wilms' tumor focused on dactinomycin. Eleven of 39 children (28%) with metastatic disease achieved complete or partial responses with this drug (3–5). In patients with nonmetastatic disease, an initial report suggested that dactinomycin increased 2-year disease-free survival to 70% (6). In a subsequent study (7), the combination of dactinomycin plus vincristine increased disease-free survival when compared to either agent alone.

We investigated the combination of doxorubicin plus vincristine for treatment of Wilms' tumor. This combination was chosen instead of adding doxorubicin to vincristine plus dactinomycin because: (*a*) complete or partial responses of metastatic Wilms' tumor are more frequent with doxorubicin (60%) or vincristine (54%) than with dactinomycin (3–5,8–17) and (*b*) doxorubicin and dactinomycin have overlapping toxic effects whereas doxorubicin and vincristine do not. Results in our series suggest that doxorubicin plus vincristine is an effective, well-tolerated therapy for Wilms' tumor and should receive broader clinical trial.

PATIENTS AND METHODS

From June 1976 to October 1981, 31 patients with Wilms' tumor were entered in the study. This represents all patients with Wilms' tumor seen during that interval, except for one child with stage IV disease whose parents refused therapy. Preoperative evaluation included chest X-rays, lung tomograms, iv pyelogram, liver-spleen scan, bone scan, cbc, BUN, creatinine clearance, and liver function tests.

To maximize exposure, the involved kidney was removed through a thoracoabdominal incision unless the tumor was small or located in the inferior pole. A lung biopsy was done if patients had a thoracoabdominal incision. A bone marrow aspirate or biopsy was done at surgery in all patients.

Tumors were staged according to the National Wilms' Tumor Study (NWTS) staging system (7). Pathologic review followed guidelines set by Lawler et al (18) and the NWTS group (19).

Chemotherapy began within 6–10 days of surgery.

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[2]Supported in part by the MACC fund and by Public Health Service grants CA-17700 and CA-17997 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Midwest Children's Cancer Center, Milwaukee Children's Hospital, and the Departments of Pediatrics (*B. Camitta, S. Adair,* and *D. Pinkel*), Radiation Therapy (*L. Kun*), Surgery (*M. Glicklich*), and Pathology (*H. Oechler*) of the Medical College of Wisconsin, Milwaukee.

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Vincristine (1.5 mg/m^2) was given weekly × 6, then every other week for 22 more doses. In the original protocol, doxorubicin was given at Weeks 1 (60 mg/m^2) and 4 (30 mg/m^2), then every 4 weeks (60 mg/m^2) for six more doses. However, because of cardiac toxicity in one patient and concerns about doxorubicin-irradiation interactions in other tissues, doxorubicin doses were reduced to 30 mg/m^2; thus, most patients received a cumulative dose of 240 mg/m^2 administered in eight equal doses. Doses of chemotherapy occasionally were delayed but rarely were decreased for neurologic or hematologic toxic effects.

Radiation therapy initially followed the guidelines of Cassady et al (1). Patients with grossly resected tumors received 2400–2600 rad in 100–150-rad fractions to the "tumor bed" (the entire region of initial tumor involvement and adjacent retroperitoneal lymph nodes, including the full width of the vertebral column). After results of NWTS I and NWTS II were established, radiation therapy was omitted for patients with stage I disease (7,20). Preoperative or intraoperative intraperitoneal spill was treated by 1900–2000 rad in 100–150-rad fractions to the whole abdomen (blocking the uninvolved kidney at 1400 rad), then boosting the tumor bed to doses as above. Pulmonary metastasis was treated by 1650–1800 rad of whole-lung irradiation in 100–150-rad fractions followed by a 1200–1400-rad boost to isolated nodules.

Follow-up included physical examinations, chest X-rays (monthly for 2 years, then at increasing intervals), liver-spleen scan (at 12 and 24 months), bone scan (at 12 and 24 months), echocardiography, blood cell counts (monthly during chemotherapy), SMA-12 (every 3 months during chemotherapy), and creatinine clearance (at 3 and 12 months). Failure of therapy was recorded for relapse at any site or for therapy-related death.

RESULTS

Patient age and sex and tumor stage and histology were typical for patients with Wilms' tumor. These parameters and details of therapy are presented in table 1.

Among 28 patients with stage I–III disease at diagnosis, there have been two treatment failures (one stage I and one stage III). Both patients had tumor histologies associated with a poor prognosis (19) and died despite salvage therapy with dactinomycin and irradiation. However, two other patients with unfavorable histologies remain free of disease 31 + and 33 + months after the beginning of therapy.

Two of three patients with stage IV disease at diagnosis remain free of disease (10 + and 24 + months after the beginning of therapy). The one patient who failed developed brain metastases after 8 months of treatment. After initially responding, the brain lesions became refractory to further irradiation and chemotherapy. At autopsy, there was no evidence of extracranial tumor.

Despite careful cardiac monitoring, one child developed congestive heart failure and died. Her cumulative doxorubicin dose was only 445 mg/m^2, but the abdominal radiation port included a portion of her heart. After the maximum allowable cumulative doxorubicin dose was lowered to 240 mg/m^2, no other child developed cardiomyopathy. The only other significant toxic effect was intestinal obstruction in three patients, two of whom had received total abdominal irradiation. A similar incidence of this complication has been reported in young children receiving extensive postoperative irradiation plus chemotherapy (21). Possible potentiation of radiation by doxorubicin in our cases cannot be ruled out.

DISCUSSION

Dactinomycin has been considered to be the mainstay of adjuvant chemotherapy for Wilms' tumor. A 70% (37 of 53 patients) 2-year disease-free survival rate was reported by Farber (6). However, there were no concurrent controls, and disease stage was not clear. In NWTS I, the 2-year disease-free survival rate following dactinomycin plus tumor bed irradiation was 83% for stage I and 57% for stages II + III (7). In an International Society of Pediatric Oncology (SIOP) study, the 2-year disease-free survival rate with dactinomycin plus tumor bed irradiation was 56% for stage I–III patients (22). Furthermore, although multiple courses of dactinomycin resulted in greater disease-free survival rates than a single course (72% versus 43%, respectively) in an early study (23), this was not confirmed in the larger SIOP study (58% with multiple courses versus 54% with a single course) (22). On balance, dactinomycin appears to increase disease-free survival by only 10% when added to surgery plus irradiation therapy for stage I–III Wilms' tumor (24).

Vincristine is more active than dactinomycin against metastatic Wilms' tumor (4,14–17). However, in NWTS I, the 2-year disease-free survival rate for stage II–III disease was 55% with vincristine and 57% with dactinomycin (7). In another study, all patients with stage I–III Wilms' tumor received one course of dactinomycin and were then randomized to receive further dactinomycin or vincristine (25). The 2-year disease-free survival rate was 54% with dactinomycin and 79% with vincristine ($P = 0.008$). Finally, when vincristine plus dactinomycin was used for stage II–III patients, the long-term disease-free survival rate was 81% in NWTS I and 65% in NWTS II (70% for both studies combined). One possible interpretation of these data is that vincristine and dactinomycin act synergistically against Wilms' tumors. Alternatively, dactinomycin may be relatively inactive, the above results reflecting primarily vincristine activity.

TABLE 1.—Patient characteristics, therapy, and clinical outcome

Patient No.	Age (yrs)	Sex	Stage (NWTS)*	Histology†	Tumor bed (other) radiation therapy‡	Total doxorubicin dose (mg/m^2)§	Mos of continuous remission	Outcome, other
1	5	F	I	PD	+	421	71+	
2	14	M	I	WD	+	357	66+	
3	3	M	I	MD	+	270	60+	
4	3	F	I	MD	0	240	49+	Hemihypertrophy
5	2	M	I	Clear cell, PD	+	[240]	[21]	Bone metastasis; died (44 mos)
6	2	M	I	WD	+	240	38+	
7	3	F	I	MD	0	240	32+	
8	1	M	I	WD	0	240	17+	
9	1	F	I	WD	0	240	7+	Uncle had Wilms' tumor
10	5	F	II	MD	+	[445]	[10]	Fatal cardiomyopathy; no tumor at autopsy
11	8	M	II	MD	+	288	59+	
12	2	F	II	MD	+	240	46+	Concomitant renal adenoma
13	1	M	II	MD	+	240	37+	
14	5	F	II	WD	+	240	34+	
15	4	F	II	Clear cell, PD	+	240	31+	
16	4	F	II	WD	+	240	31+	
17	4	M	II	MD	+	240	26+	
18	1	F	II	MD	+	240	21+	Multifocal intrarenal tumors
19	3	M	III R	PD	+ (WA)	297	57+	
20	10	M	III ¶	Anaplastic, PD	+ (WA)	[205]	[8]	Lung metastases; died (23 mos)
21	6	F	III S	MD	+ (WA)	240	43+	
22	11	F	III S	Anaplastic, PD	+ (WA)	240	33+	Rhabdoid elements
23	5	F	III S	MD	+ (WA)	240	32+	
24	2	M	III	MD	+	240	29+	
25	1	F	III S	PD	+ (WA)	240	17+	Nodes, positive
26	1	M	III R	MD	+ (WA)	240	16+	
27	3	M	III	WD	+	240	9+	Local spill
28	3	F	III R	MD	+ (WA)	240	7+	Invasive
29	8	F	IV S	PD	+ (WA, WL)	240	[8]	Brain metastases; died (19 mos), CNS tumor only at autopsy
30	5	M	IV	WD	+ (WL)	240	24+	
31	2	F	IV	WD	+ (WL)	240	10+	

*S = operative spill; R = preoperative rupture.
†WD, MD, PD = well, moderate, or poor tubular differentiation and not clear cell or anaplastic. *See* refs 18 and 19 for details.
‡WA = whole abdomen; WL = whole lung.
§Dose at failure is shown in brackets.
¶Inoperable. Biopsied elsewhere. Received doxorubicin (× 1) and vincristine (× 2) at Milwaukee Children's Hospital before resection.

Doxorubicin also is more active than dactinomycin against metastatic Wilms' tumor (8–13). In NWTS II, when doxorubicin was added to vincristine plus dactinomycin for stage II–III tumors, long-term disease-free survival rates increased from 65% to 80% (20). In our study, 17 of 19 (89%) children with stage II–III disease remain alive and disease-free after treatment with doxorubicin plus vincristine. These data suggest that doxorubicin is more effective than dactinomycin for adjuvant therapy of Wilms' tumor. Alternatively, our excellent results might be due in part to a more effective time-dose schedule of doxorubicin and vincristine in addition to substitution of doxorubicin for dactinomycin per se. More intensive dactinomycin-vincristine is being compared to doxorubicin-dactinomycin-vincristine in NWTS III. Given equivalent antitumor efficacy and short-term toxicity, a regimen not utilizing doxorubicin would be preferable because of less potential long-term cardiac toxicity.

In NWTS II, the disease-free survival rate of children with stage I disease was 90% after 6 or 15 months of dactinomycin-vincristine. One-half of the relapses occurred in patients whose tumors had unfavorable histologies (20). Despite similar results using doxorubicin-vincristine in our study, dactinomycin-vincristine is preferable for children with favorable-histology stage I disease, because of decreased potential cardiac toxicity.

Too few patients with stage IV disease or unfavorable histologies were treated in this study to make meaningful comparisons with previous regimens. Current therapy may cure 40%–60% of children presenting with

stage IV disease (1,2) and 30%–40% of children whose tumors have unfavorable histologies (2,19,20). Doxorubicin-vincristine as utilized in this pilot study deserves further trials in children presenting with these types of Wilms' tumors.

Our data suggest that doxorubicin may be more effective than dactinomycin in the treatment of Wilms' tumor. At a maximum cumulative doxorubicin dose of 240 mg/m^2, clinically apparent cardiac toxicity is extremely rare (26). However, even low doses of doxorubicin may cause microscopic cardiac damage (27). The long-term consequences of these subclinical lesions are not known. Therefore, caution is suggested before results in this study are applied to larger groups of patients with Wilms' tumor.

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Invited Comment on the Milwaukee Pilot Study of Doxorubicin-Vincristine Therapy for Wilms' Tumor

Dr. Camitta and colleagues (1) report very impressive results in a study of Wilms' tumor in a single institution. Thirty-one patients representing all stages of the disease, four of them with unfavorable histologic features, were treated with a regimen that included doxorubicin (ADR) and vincristine (VCR). Twenty-seven of the 31 children survive for periods ranging from 2 to 67 months (median follow-up time, 28 months). Two of the four failures occurred in patients with tumors of unfavorable histology, one died of cardiotoxicity, and one had metastases at diagnosis.

The authors present a brief but complete review of the literature concerning ADR therapy for Wilms' tumor, present their rational for the use of this agent (which they consider to be superior to dactinomycin [AMD]), and attribute the good results reported to the use of ADR. However, there are other possible explanations for the better results in these patients.

Camitta and his colleagues gave both ADR and VCR more frequently than they have generally been given in the large cooperative studies. Thus, the Milwaukee investigators administered VCR weekly for 6 weeks, and then every other week for 22 additional doses. This is to be contrasted with the way VCR was used in the first and second National Wilms' Tumor Studies (NWTS I and II) and by the International Society of Pediatric Oncology, where it was given every 3 months during the maintenance phase. The United Kingdom Medical Research Council results, mentioned by Camitta and colleagues, found that VCR every other week gave results superior to those with the 3-month schedule used in NWTS I. AMD, too, was given every 3 months in NWTS I and II, rather than on a monthly schedule such as that employed in Milwaukee. Green et al (2) found that AMD appeared to be more efficient in leukemic patients when given monthly than when given in the more traditional 3-month cycles. Similar observations were recorded by Green (3) for children with solid tumors given AMD, VCR, and cyclophosphamide in relatively high-dose pulses.

Therefore, it is equally plausible that the good results reported by Camitta et al are secondary to a more efficient use of effective agents, rather than to the ADR itself. This hypothesis is being put to the test in the third NWTS, where both AMD and VCR are being given more frequently than before (4), although the schedule as well as the drugs are different from those used by Camitta et al. Preliminary data from NWTS III do not suggest any major difference between AMD and VCR used more intensively versus the three-drug regimen (AMD + VCR + ADR) that gave the best overall results in NWTS II.

Thus, while the Milwaukee results are impressive, it remains to be shown that it is the ADR rather than the scheduling differences that are responsible. These considerations would matter relatively little if it were not for the fact that even modest doses of ADR are associated with demonstrable myocardial damage (5). Thus, while the total dose of ADR used in Milwaukee seems to be safe on the basis of short-time observations, the long-term effects—measured in decades and scores of years in surviving children—are unknown and are reasons for concern. Indeed, it is this latter consideration which led the NWTS group to test the more intensive AMD + VCR regimen against the one that included ADR as well. The object was to avoid the use of ADR as a routine in these young children if it could be shown that elimination of the drug did not significantly affect relapse-free survival.

Camitta et al are correct in stating that the objective in pediatric oncology is to obtain relapse-free survival in as many patients as possible, but the cost in terms of toxicity in achieving such "coverage" needs to be put in the balance. NWTS II has shown that 88% of group I children survive relapse-free 2 years beyond the date of surgery when they are given AMD + VCR (without radiation therapy) postoperatively. Of children with group II or III Wilms' tumor of favorable histology, 70% survive free of disease $\geq$ 2 years after postoperative irradiation, AMD, and VCR. Addition of ADR to the regimens for these patients means that seven or more of ten children with locoregional tumors of favorable histology would be given the cardiotoxin unnecessarily. Camitta and his colleagues are therefore well-advised when they caution against indiscriminate trials of ADR in patients with Wilms' tumor.

The Milwaukee data should encourage investigators to try different schedules of drugs of known or marginal effectiveness, not only in Wilms' tumor but in other tumor systems as well. The report is also of interest because the total treatment time is shorter than that in several large studies. It may well be that the more efficient use of effective chemotherapeutic agents not only will give better results but also will shorten the

overall time that treatment must be administered to patients. This is a gain of considerable importance in itself.

Finally, the article is important in another way. It demonstrates that there is ample room for carefully planned and well-conducted clinical investigations in single institutions. This pertains even in the study of neoplasms that appear to be dominated by cooperative clinical trials such as the NWTS and studies by the International Society of Pediatric Oncology and the Medical Research Council.

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Treatment of Advanced Prostate Cancer With Cyclophosphamide, Doxorubicin, and Methotrexate[1,2,3]

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Twenty-three patients with advanced prostate cancer who had failed previous hormone therapy were treated with cyclophosphamide, doxorubicin, and methotrexate on a 3-week course. Of the 22 evaluable patients, over one-half had poor performance status, increased acid and alkaline phosphatase levels, and pain. Parameters which improved in > 50% of cases included acid and alkaline phosphatase levels, pain, performance status, and measurable lesions (lung and soft tissue). Initial parameters associated with a significantly decreased survival were age > 66 years, increased pain, poor performance status, and increasing alkaline phosphatase. Prior radiation therapy was associated with increased drug toxicity, lower doses of chemotherapy, and decreased survival (not significant). There was a significant relationship between the degree of improvement of acid phosphatase, alkaline phosphatase, pain, and performance status to increased survival. Three categories of response were defined based on these parameters. The mean survival of seven patients with partial response (106 weeks) is significantly longer than that of seven with measurable response (57 weeks) and eight with no response (26 weeks). Four patients had severe leukopenia and one died of sepsis. These results compare favorably with previous reports of chemotherapy treatment of advanced prostate cancer. [Cancer Treat Rep 66:1797–1802, 1982]

Only recently has there been an emphasis on the treatment of advanced prostate cancer with combination chemotherapy (1). Drugs with activity include cyclophosphamide (Cytoxan; Cyt), doxorubicin (Adriamycin; Adr), 5-FU, and methotrexate (MTX) (1,2).

Several studies (3–6) have included the combination of Cyt and Adr because of its efficacy in other solid tumors (7). There is evidence that Cyt is more effective when given in high intermittent doses than as daily oral therapy (8,9). In this study, Cyt and Adr were administered on Day 1 followed by four biweekly doses of MTX beginning 8 days later. There are cytokinetic data in other solid tumors (10,11) to suggest an advantage to administration of an S-phase drug like MTX when recruitment of cells into DNA synthesis has occurred. A similar schedule of therapy was shown to be advantageous for lung cancer (10).

A standard nomenclature for response criteria was developed by the National Prostatic Cancer Project (2). However, many recent studies have defined their own criteria (3,4,12,13), in part due to the need for categories that seem more relevant to results now being achieved. Advanced prostate cancer has important features such as a high percentage of positive bone scans and acid and alkaline phosphatase levels, but few patients have measurable lesions.

In the present study we have analyzed a series of pretreatment variables and their relation to survival. Several parameters have been serially studied and related to response and survival. The Cyt, Adr, and MTX (CAM) regimen has induced a high percentage of remissions and improved survival and, as a consequence, it has been possible to determine several parameters associated with a better prognosis.

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MATERIALS AND METHODS

From April 1976 to December 1979, 23 patients with stage D prostatic carcinoma were entered in this protocol. One patient who developed a spinal cord compression prior to receiving the second course of therapy was excluded from analysis. Patients were excluded with an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 4, or a cardiac history or ECG contra-indicating the use of Adr. Most patients had received previous treatments (table 1). Patients who had had a prior orchiectomy and/or treatment with hormones had evidence of disease progression. No patient had received previous chemotherapy. Of the 12 patients who had had prior radiation, eight had received 5000–8000 rads to the prostate and pelvis, and one of these had also received radiation to the peri-aortic area. Four patients had had radiation to sites of bony metastases: hip, ribs, and spine.

The data of the 22 evaluable patients were analyzed as of April 1981. The mean age was 69 years (range, 52–83). All patients were evaluated with: complete history and physical examination, cbc and platelet count, blood chemistries, serum prostatic acid phosphatase, urinalysis, chest X-ray, IVP, and bone scan. Most patients had a liver scan, a bone marrow biopsy and aspirate, and a serum carcinoembryonic antigen (CEA).

The ECOG PS scale (0–4) was used. Pain levels and urinary obstructive systems were graded on a 4-point (0–3) scale of increasing severity.

Histology sections were classified as well, moderately, or poorly differentiated.

Measurable chest X-ray and subcutaneous lesions were determined by caliper, the longest diameter times its perpendicular.

Physical evaluation, blood chemistries, and analysis of subjective parameters were repeated every 3 weeks. Chest X-rays were repeated every 6–9 weeks, if abnormal. The bone and liver scans and IVP were repeated every 3 months, if abnormal. Bone marrow determinations were not repeated. The liver scan was accepted as abnormal if there were distinct multiple defects.

TABLE 1.—Previous treatment of 22 patients receiving CAM treatment for prostate cancer

Previous treatment	No. of patients
Prostatectomy	4
TURP*	16
Orchiectomy	15
Radiation	12
Hormonal	17

*Transurethral resection of the prostate.

All patients began treatment with a normal wbc and platelet count and creatinine of < 1.6 mg/100 ml. Treatment consisted of 600 mg/m^2 iv of Cyt and 40 mg/m^2 iv of Adr on Day 1, and 15 mg/m^2 orally of MTX on Days 9, 13, 16, and 20. The courses were repeated every 3 weeks and the doses were adjusted as shown in table 2. After an episode of severe leukopenia following the first treatment in two patients who had had prior radiation, initial treatments were reduced to two-thirds for this group.

The parameters that were determined were divided into objective (table 3) and subjective (table 4) parameters. For the abnormal blood tests, a complete response (CR) was defined as normalization of the laboratory value. A partial response (PR) was defined as a 50% decrease in an abnormal value but the decreased value remained above normal limits. A CR for measurable lesions, liver scan, or bone scan required normalization. A PR for measurable lesions and liver scan defects was a 50% decrease in the product of the largest diameter and its perpendicular.

For subjective parameters, a CR represented normalization, eg, to PS 0. A PR was a clear improvement of at least one grade.

In the overall analysis, establishing definitions of response is more complicated. In this and other studies, an abnormal bone scan or enlarged prostate rarely nor-

TABLE 2.—Dose modifications of CAM

Blood count (cells/mm^3) on Day 1 of new treatment course		Dose modifications		
Wbcs	Platelets	Cyt	Adr	MTX
≥ 4000	≥ 100,000	Increase 10%	Increase 10%*	No change
3000–4000	≥ 100,000	No change	No change	No change
2000–3000	≥ 100,000	Give 2/3 dose	Give 2/3 dose	No change
≥ 3000	75,000–100,000	No change	Give 2/3 dose	Give 2/3 dose
≥ 3000	50,000–75,000	Give 2/3 dose	Omit†	Omit
2000–3000	≥ 50,000	Give 1/3 dose	Omit	Give 1/3 dose
≤ 2000	≤ 50,000	Omit	Omit	Omit

*Adr should not exceed 60 mg/m^2.

†After dosage omission, resume next dose at 2/3 of offending dose of wbcs ≥ 3000/mm^3 and platelets ≥ 100,000/mm^3.

TABLE 3.—Objective parameters of 22 patients receiving CAM treatment for prostate cancer

Parameter	No. of patients tested	No. abnormal	No. of —		% response
			CR	PR	
Measurable*	22	7	5	2	100
Alkaline phosphatase	22	19	9	5	74
Acid phosphatase	22	12	3	4	58
CEA	18	9	2	3	56
Liver scan	18	5	1	1	40
Large prostate	22	9	0	3	33
Bone scan	22	22	1	0	5
Bone marrow	13	6	—	—	—

*8 lesions in 7 patients: 3 lung nodules, 2 abdominal masses, 1 node, 1 subcutaneous lesion, 1 retro-orbital mass. Patient with 2 lesions, both responding, was considered a PR.

malized. We therefore defined a PR as complete normalization of all abnormal objective parameters except an abnormal bone scan or enlarged prostate. A measurable response (MR) was defined as a 50% improvement in all objective parameters, except bone scan and enlarged prostate. All other patients had no response (NR). These definitions provide the maximum consistency with definitions recently used by others (1,3–7). We have attempted in effect to define three categories of overall response. The labels PR, MR, and NR are not ideal descriptors.

RESULTS

Twenty patients were white and two were black. The majority of patients had previously received several types of treatment (table 1). Ten patients had PS 0 or 1, and 12 had PS 2 or 3.

The parameters and responses are shown in tables 3 and 4. Bone scans were abnormal in all patients. Pain, abnormal PS, and an elevated alkaline phosphatase each occurred in 19 of 22 patients. Acid phosphatase was elevated in 55%.

There were eight measurable lesions noted in seven patients which included a retro-orbital mass and lung nodule in one patient. There were two other lung lesions, one subcutaneous lesion, one node, and two abdominal-pelvic masses. In all seven patients there was a response: five CR and two PR. The patient with retro-orbital disease had marked proptosis that normalized and was considered a PR. Alkaline phosphatase objectively improved in 74% of patients and acid phosphatase improved in 58%. Bone scan objectively improved in only one patient. There was significant improvement of pain in over three-fourths of the patients and an improved PS in over one-half.

Survival data according to response are shown in table 5. The overall mean survival was 61 weeks. The median survival was 47 weeks, with two patients alive. The patients with PR lived significantly longer than those with MR ($P < 0.001$) or NR ($P < 0.001$), and those patients with MR in turn lived significantly longer than those with NR ($P < 0.05$).

There was no apparent influence of histologic grade on survival. The type of previous hormonal treatment or surgery did not influence survival.

The factors which may have influenced survival are shown in table 6. Patients ≤ 66 years old survived longer than older patients ($P < 0.01$). An analysis of variance demonstrated a significant trend of increasing age and decreased survival ($P < 0.01$). Patients with little or no pain (grades 0 or +1) survived longer than those with grades +2 or +3 ($P < 0.10$). Prior radiation was associated with reduced survival ($P = 0.13$), and patients who had PS 0 or 1 survived longer than those who had PS 2 or 3 ($P < 0.05$).

Factors which did not influence survival were initial CEA, abnormal liver scan, measurable tissue lesions, urinary obstructive symptoms, and positive bone marrow. Prior surgery had a negative effect, but it was not significant.

An analysis of variance demonstrated no significant relationship between initial acid phosphatase and sur-

TABLE 4.—Subjective parameters of 22 patients receiving CAM treatment for prostate cancer

Parameter	No. abnormal	No. of —		% response
		CR	PR	
Pain	19	9	6	78
PS	19	6	5	58
Urinary obstruction	11	4	1	45

TABLE 5.—CAM treatment of prostate cancer: response

Response	No. of patients		Survival in wks	
	Total	Alive	Mean (range)	Median
PR	7	1	106 (41–258+)	90
MR	7	1	57 (17–108)	57
NR	8	0	26 (15–45)	21
Total	22	2	61 (15–258+)	47

vival, although the two survivors are in the normal acid phosphatase group. Few patients had normal alkaline phosphatase values. There was a significant trend of increasing values and poorer survival ($P < 0.05$).

The extent of response of acid phosphatase, alkaline phosphatase, pain, and PS was related to survival; for these parameters, the survival was normal > CR > PR > NR. For alkaline phosphatase and PS, this trend of decreasing survival was significant at $P < 0.01$ and $P < 0.001$, respectively. Survival of CR was significantly greater than NR for all groups and greater than PR for pain and PS. Improvements of measurable lesions, CEA, scans, and urinary obstruction were too infrequent for a similar analysis.

The average survival from diagnosis to beginning this treatment was 144 weeks (range, 2–596). There was no relationship between this pretreatment interval and posttreatment survival. The average survival from diagnosis to treatment for patients with PR, MR, and NR was 101, 155, and 171 weeks, respectively (not significant). Similarly, there was no relationship to any pretreatment parameters.

This treatment resulted in a nadir wbc count < 1000/mm^3 in four patients, sepsis in three patients, and death in one patient. All four patients had received prior radiation.

To determine the possible effect of pretreatment parameters on drug dose and leukopenia, an analysis was made of the nadir leukopenia and doses of Cyt and Adr during the third course of treatment. A comparison of the lowest wbc count for each patient would be biased by the fact that patients who received more courses and survived longer had greater opportunity for lower blood cell counts. By the third course of treatment, patients started at lower initial drug doses due to previous radiotherapy could have been dose-incremented to full doses if blood cell counts permitted. Seventeen patients were evaluable. There were no statistically significant differences in nadir wbc count or dose of Cyt and Adr based on pretreatment parameters. Patients who had had previous radiation had a lower wbc count ($P < 0.10$) and received less Cyt ($P < 0.10$) than patients with no previous radiation. There were too few patients to make a distinction between those who received a major course of radiation to the pelvis (≥ 5000 rads) and those whose radiation was to metastatic sites only. There were no differences based on categories of response.

Mucositis occurred frequently and MTX was decreased progressively until the mucositis no longer occurred. Alopecia was universal. Adr was discontinued in one patient who developed early congestive heart failure after eight courses. The patient responded to

TABLE 6.—CAM treatment of prostate cancer: relation of age, pain level, previous radiation, and PS to survival

Parameter	No. of patients	No. alive	Survival in wks	
			Mean	Median
Age in yrs				
≤ 66	7	1	103	95
67–72	8	1	49	48
≥ 73	7	0	33	22
Pain level				
0–+1	8	2	94	81
+2	6	0	44	48
+3	8	0	41	32
Previous radiation				
No	10	2	81	50
Yes	12	0	44	37
PS				
0, 1	10	2	87	58
2, 3	12	0	37	32

diuretics and digitalis. One patient died of a myocardial infarction. The other deaths appeared to be related to the cancer.

DISCUSSION

In order to devise meaningful criteria for the evaluation of response in prostate cancer one must be able to follow various parameters serially, and the treatment must be relatively effective. Yagoda et al (13) discussed the advantages of selecting patients with measurable disease for study. In prostate cancer, patients with measurable lesions may not be a representative group. In this study 32% of patients had measurable lesions, all of whom responded to chemotherapy.

We divided the parameters into objective and subjective categories. Other studies have distinguished between direct and indirect parameters (4). Both systems are at best artificial. The important issue is to determine the prognostic value of the initial determination and the relation of a change in the parameter to survival (14).

The prostate size is not generally a useful parameter since so many patients undergo prior transurethral resection of the prostate and/or radiotherapy. The bone marrow is frequently positive but serial testing may be inconclusive.

Aside from a minority of patients with measurable or evaluable lesions, the study of response is restricted primarily to chemical, X-ray, and subjective measurements.

We defined changes of parameters in standard fashion, ie, CR = normalization. Our group of patients had a particularly poor prognosis, having all failed previous hormone therapy and 12 of 22 having PS 2 or 3. Typically, bone scans were positive and pain was frequent and often severe.

Our analysis indicated that younger patients have a significantly better prognosis (table 6). This is in accord with Berry et al (15) but not with Yagoda et al (13) or Eagan et al (12). The initial PS in our study was significantly related to survival (table 6). This has been noted by most investigators (13,15). Ihde et al (4) restricted their study to ambulatory patients.

Similarly, we found that the initial value for alkaline phosphatase correlated inversely with survival but the correlation for acid phosphatase was not significant. Berry et al (15) and Johnson et al (14) documented the significance of acid phosphatase in larger series.

We found that patients with previous radiation had appreciably decreased survival times. This relationship was noted by Schmidt et al (2) and DeWys et al (16) and as in our study seems to be due to reductions in chemotherapy dose in patients with prior radiation. In Berry's study (15) there was no relationship between prior radiation and survival. However, the chemotherapy used caused little myelosuppression.

Other initial parameters which may be associated with a poorer prognosis include: positive bone marrow, elevated CEA or LDH, decreased hemoglobin, prior surgery, poorly differentiated tumor, and positive liver scans (2,14,15,17,18). In our study of 22 patients these factors were not significant, which may in part reflect inadequate numbers of cases.

We found that improvement in acid or alkaline phosphatase, pain level, or PS significantly correlated with a better survival. Johnson et al (14) noted a similar correlation for acid phosphatase and pain but not for PS. Bone scans are not useful in the assessment of response since responses are as yet rarely, if ever, noted.

An overall assessment of response can be made once individual parameters are analyzed. We have been able to define three separate categories of overall response. In this study we defined PR, MR, and NR as stated. This is not unlike the approach of Ihde et al (4). In this study, the average survival of patients determined to have the best response (PR) was > 2 years compared to just > 1 year for MR patients and $^1/_2$ year for NR patients. Other definitions of response have proved useful (2–4,13). With further improvement in treatment results more exact criteria will be possible.

In this study, three drugs were used in a moderately toxic regimen. Many investigators heretofore have been especially leery of using doses of drugs in this disease which are comparable to those used in other diseases. Nevertheless, good response rates in this disease as in most other solid tumors will probably require adequate levels of drugs.

The response rate and survival in this study compare quite favorably with previous reports of combination chemotherapy (1). The overall survival exceeded 1 year, with approximately one-third of the patients living 2 years.

In most reports, patients previously treated with hormones survive < 30 weeks. Several recent reports have included the use of Cyt and Adr. Izbicki et al (3) reported a 17- and 40-week survival for nonresponders and responders, respectively. In a study restricted to ambulatory patients, the overall survival was 11 months (4). In a third study, average survival was not given (5). In this study, the survival of ambulatory patients was 20 months. Single agents have been far less effective.

The apparent improved results achieved may be due to somewhat higher drug doses. In addition, the use of the cycle-specific drug MTX may be important. In lung cancer the response rate is approximately doubled by addition of MTX in a similar schedule following pulse Cyt (19). MTX adds little to the hematologic toxicity as given, but does cause mucositis.

It is likely that further progress in prostate cancer will be made using combinations of drugs with the same vigor and fundamental designs that have been successful in other cancers. It will be important to develop a uniform nomenclature of response so that reports will be comparable. In the meantime, detailed testing and reporting of results will help elucidate the directions for further studies.

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Treatment of Advanced Colorectal and Gastric Adenocarcinomas With 5-FU Combined With High-Dose Folinic Acid: A Pilot Study[1]

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Thirty patients with advanced colorectal adenocarcinoma and five with advanced gastric adenocarcinoma were studied; all had measurable tumor. The treatment used was based on biochemical and cell culture studies which have demonstrated that an excess of intracellular reduced folates is necessary to provide optimal inhibition of thymidylate synthetase and to increase the cytotoxic effect of fluorinated pyrimidines. The treatment comprised 5-FU (370–400 mg/m²/day) and high-dose folinic acid (200 mg/m²/day) given simultaneously for 5 consecutive days, with a 21-day interval between treatments. Of the 30 patients with advanced colorectal carcinoma, 16 had not been previously treated with cytostatic agents and 14 were resistant to previous chemotherapy with 5-FU given either as a single agent or combined with other drugs. The response rates, both complete and partial, were 56% and 21% in the previously untreated and the previously treated patients, respectively. The five patients with advanced gastric adenocarcinoma were previously untreated; three achieved a partial response. The overall toxicity was acceptable. The most prominent side effect was oral mucositis; myelosuppression and diarrhea were less prevalent. Severe toxicity, when present, was prevented in subsequent courses by a 30-mg/m² reduction in the daily dose of 5-FU. We conclude that in these types of tumors, folinic acid at high doses increases the effectiveness of 5-FU given simultaneously and renders some colorectal tumors, previously resistant to 5-FU, sensitive to this drug. [Cancer Treat Rep 66:1803–1807, 1982]

The use of 5-FU as a single agent in the treatment of advanced colorectal and gastric adenocarcinomas has been disappointing; responses, generally of short duration, have been observed in 20% of the patients with colorectal carcinoma and in 25% of the patients with gastric carcinoma, but there has been no proven increase in the overall survival rate (1–3).

The rationale of the clinical trial reported here is based on the results of biochemical and cell culture studies performed with various cell lines (4–7), demonstrating that an excess of intracellular reduced folates is necessary for optimal inhibition of thymidylate synthetase (TS) and for an increased cytotoxic effect of fluorinated pyrimidines. It was our aim to enhance the activity of 5-FU in colorectal and gastric adenocarcinomas by simultaneous administration of folinic acid (FA) in high doses (8).

PATIENTS AND METHODS

Patients entered in this study had histologically confirmed colorectal or gastric adenocarcinomas; all had metastatic disease.

Patients with colorectal adenocarcinoma.—Thirty-five patients were entered in the study; five were excluded because their tumors could not be measured accurately. The 30 evaluable patients had measurable disease; 23 had histologically proven metastases, and seven had metastatic tumors as evidenced by radiologic, ultrasonographic, and/or scintigraphic proce-

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TABLE 1.—Patient characteristics and response

Patient No.	Age (yrs)	Sex	Prior treatment with 5-FU*	Tumor site	No. of courses at evaluation	Response to therapy†	CEA titer before/after therapy (ng/ml)†	Response site	Duration of response or disease stabilization (days)
				Patients with colorectal adenocarcinoma					
1	50	F	—	Pelvis, vagina	9	CR	7.8/2.5	Pelvis, vagina	258+
2	58	M	—	Liver	9	PR	99/47	Liver	258+
3	59	M	—	Liver	7	PR	2.5/2.5	Liver	197+
4	55	F	—	Liver	8	PR	64/55	Liver	213+
5	50	M	—	Liver, lung (nodular)	8	PR	13/<2.5	Liver, lung	229+
6	58	F	—	Liver, abdominal tumor	6	PR	11.7/5.4	Liver, abdomen	136+
7	75	F	—	Nodes (peripheral), abdominal tumor	5	PR	ND	Nodes, abdomen	122+
8	37	M	—	Liver, nodes (peripheral), abdominal tumor	6	PR	57/32	Nodes, abdomen	183+
9	61	F	—	Liver	4	PR	35/31	Liver	92+
10	51	F	—	Liver, adrenals	8	NC	52/32	—	197
11	71	M	—	Liver	14	NC	28/15	—	365
12	50	M	—	Liver	10	NC	800/830	—	244+
13	47	M	—	Liver	5	PD	201/210	—	0
14	70	M	—	Liver, neoplastic ascites	3	PD	120/200	—	0
15	61	M	—	Liver, neoplastic ascites	2	PD	ND	—	0
16	45	M	—	Liver	3	PD	64/75	—	0
17	48	M	(a)	Liver	13	CR	19/<2.5	Liver	318+
18	37	M	(b)	Liver	5	PR	60/7.5	Liver	136+
19	56	M	(c)	Liver	6	PR	1205/292	Liver	152
20	49	M	(d)	Liver	8	NC	1030/92	—	228+
21	52	F	(b)	Liver	4	NC	22.2/19.3	—	90+
22	53	M	(e)	Liver	6	NC	550/200	—	153+
23	46	F	(e)	Liver	9	NC	18.8/17	—	305+
24	51	M	(f)	Liver	10	NC	23/35	—	244
25	41	M	(g)	Liver, nodes (peripheral)	3	PD	5/4	—	0
26	72	M	(g)	Bone, abdominal tumor	5	PD	14/16	—	0
27	60	M	(e)	Liver	3	PD	68/86	—	0
28	30	M	(g)	Liver, lung, abdominal tumor	3	PD	162/93	—	0
29	65	M	(e + h)	Liver	3	PD	ND	—	0
30	40	M	(g + h)	Liver	5	PD	100/100	—	0
				Patients with gastric adenocarcinoma					
1	69	F	—	Abdominal wall, stomach, liver	4	PR	ND	Liver, abdominal wall	90+
2	63	M	—	Liver, stomach	4	PR	70/15	Liver, stomach	90+
3	34	F	—	Liver, neoplastic ascites	2	PR	ND	Liver, ascites	60+
4	67	M	—	Nodes (mediastinal), stomach	3	NC	6.8/5	—	60+
5	71	M	—	Liver, abdominal tumor	4	PD	ND	—	0

*Previous treatment (only doses of 5-FU are given): (a) = 5-FU (400 mg/m^2/day × 5); (b) = doxorubicin + mitomycin + 5-FU (400 mg/m^2/day × 5); (c) = cyclophosphamide + mitomycin + 5-FU (400 mg/m^2/day × 4); (d) = 5-FU (400 mg/m^2/wk); (e) = cyclophosphamide + 5-FU (400 mg/m^2/day × 5); (f) = doxorubicin + cyclophosphamide + 5-FU (400 mg/m^2/day × 4); (g) = lomustine + 5-FU (400 mg/m^2/day × 4); and (h) = mitomycin + 5-FU (500 mg/m^2/wk).

†CR = complete response; PR = partial response; NC = no change; and PD = progressive disease; ND = not determined.

dures. Patient ages ranged from 30 to 75 years (mean ± SD, 53.2 ± 11). Before entering the trial, 14 patients had been subjected to an ineffective regimen of 5-FU given either as a single agent or combined with other drugs; the other 16 patients had not received previous chemotherapy (table 1).

Patients with gastric adenocarcinoma.—Six patients were entered in the study; one was excluded because neoplastic ascites was the sole indicator of disease. Five previously untreated patients with measurable disease were evaluated (table 1). Their ages ranged from 34 to 71 years (mean ± SD, 60.8 ± 15). The presence of metastases was proved histologically in all cases.

Treatment comprised 5-day courses followed by drug-free intervals of 21 days. FA was given at a dose of 200 mg/m²/day by iv bolus injection, and 5-FU was given immediately afterwards at a dose of 370 mg/m²/day by iv infusion over 15 minutes. In subsequent courses, the daily dose of 5-FU was raised to 400 mg/m² if toxicity was absent or mild at 370 mg/m²/day. The daily dose of 5-FU was reduced by 30 mg/m² in cases of granulocytopenia of < 1000 polymorphonuclear neutrophils (PMN)/mm³ complicated by fever, thrombocytopenia of < 50,000 platelets/mm³, and/or World Health Organization (WHO) grade 3 or 4 oral mucositis or diarrhea (9). Treatment courses were repeated until failure became evident or until progressive disease developed in previously responding or previously stable patients.

Before each course, each patient had a physical examination, peripheral blood cell counts including differential, liver function tests, and determinations of serum creatinine, serum electrolytes, glucose, and BUN. CEA was determined in 29 patients. Measurable disease was initially documented by physical examination and by anatomically oriented tests (chest roentgenogram, computerized tomographic [CT] scan, ultrasonography [USG], endoscopy, and/or radionuclide scan [RNS]) which were repeated during the follow-up period. Response was determined quantitatively by comparison of objectively measurable lesions and was categorized as CR, PR, no change, or progressive disease. CR was defined as the disappearance of all perceptible tumors, and PR was defined as a reduction in the sum of the products of the longest perpendicular diameters of all measurable lesions by at least 50% in the absence of any new site of malignancy. Patients in whom a 50% tumor regression could not be established and those in whom stable disease was recorded for at least 3 months were considered to have no change. Progressive disease was defined as the appearance of any new lesion and/or growth of existing lesions. Duration of response was determined from the start of therapy.

RESULTS

Patients With Colorectal Cancer

Of the 16 previously untreated patients, one achieved a CR and eight a PR. The complete responder had disappearance of pelvic and vaginal tumors as demonstrated by endoscopy and laparotomy. Of the partial responders, four patients with hepatic metastases as the sole indicator had tumor regression, which was measured in one patient by laparotomy, in two by both RNS and USG, and in one by USG. Another patient had reduction of pulmonary nodules and of liver metastases, as measured by CT scan, USG, and RNS, as well as disappearance of obstructive jaundice. One patient had regression of abdominal masses and of hepatic metastases, as measured by both RNS and USG. Finally, two patients had regression of metastatic nodes and abdominal masses. The response rate in this group of patients was 56%; responses lasted from 92+ to 258+ days (mean, 188).

Of the 14 patients previously resistant to 5-FU, one attained a CR and two a PR. The complete responder had disappearance of hepatic metastases, as evidenced by both RNS and CT scan. The two partial responders had regression of hepatic metastases, evidenced in one case by CT scan and in the other by RNS. The response rate in this group was 21%; responses lasted 136+, 152, and 318+ days. The overall objective response rate for the 30 patients with colorectal adenocarcinoma was 40%.

TABLE 2.—Myelosuppression in 16 patients who had weekly blood cell counts during 2 mos for 2 consecutive courses at each dose level of 5-FU

Course	5-FU dose (mg/m²/day × 5)	Mean nadir (range) PMN*	Mean nadir (range) Platelets†	Mean Day of nadir from Day 1 of treatment (range)	Mean time of recovery in days (range)
1	370	29.9 (8–80)	296 (140–472)	13 (12–19)	8 (7–9)
2	400	19.2 (1–64)	238 (120–350)	14 (12–19)	8 (4–14)

* × 10^2 cells/mm³.
† × 10^3 cells/mm³.

Patients With Gastric Cancer

Of the five evaluable patients, three achieved a PR which lasted for 60+, 90+, and 90+ days. Tumor regression was very rapid and resulted in a dramatic reduction of tumor size. In one patient, a large lesion of the abdominal wall disappeared; another had regression of a gastric tumor as measured by endoscopy, and in another, neoplastic ascites disappeared. All three responding patients had regression of hepatic metastases as measured by USG.

Toxicity

Myelosuppression was studied in 16 patients who had blood cell counts weekly for 2 months during two consecutive courses at two different dose levels of 5-FU; the results (table 2) indicate that myelosuppression was moderate, with rapid recovery.

The overall toxicity is indicated in table 3. The 35 patients received a total of 207 courses of therapy, of which 198 were evaluated for toxic effects. The 5-FU doses were 370 mg/m^2/day × 5 (88 courses) and 400 mg/m^2/day × 5 (110 courses). The mean number of courses per patient was six (range, two to 14). Granulocytopenia of < 2000 PMN/mm^3 was recorded in 17 courses in 13 patients. Seven of these patients had been treated previously with chemotherapy or radiotherapy. Granulocytopenia of < 1000 PMN/mm^3 occurred in seven courses; six of these were complicated by fever, which in one case was due to *Staphylococcus aureus* septicemia. The six episodes of fever occurred at the higher dose of 5-FU. Five episodes of thrombocytopenia with < 150,000 platelets/mm^3 were recorded in four patients; three episodes of thrombocytopenia with < 100,000 platelets/mm^3 occurred in three pretreated patients. There were no hemorrhagic complications. No cumulative myelosuppression was detected in comparisons of PMN and platelet counts obtained at the end of the 21-day intervals in the 26 patients who received at least four courses of treatment (table 4).

Twenty-five episodes of oral mucositis were recorded in 17 patients and were severe (WHO grade 3 or 4) in five courses at the higher dose of 5-FU; the mucositis began on Days 6–10 and persisted for 7–10 days. Eleven episodes of diarrhea were noted in nine patients and were severe in two (WHO grade 3). Partial alopecia was seen in four patients. Moderate skin rash associated with conjunctivitis was recorded in two patients. Mild nausea was observed in most patients during the treatment courses. Neither renal nor neurologic toxicity was observed. There was no death due to toxicity. Toxic effects required 5-FU dose reductions in two courses at 370 mg/m^2/day (2%) and in 11 courses at 400 mg/m^2/day (10%).

TABLE 3.—Overall toxicity in 35 patients treated with 5-FU and high-dose FA

Toxic effect and degree	No. of patients experiencing toxic effects	No. of courses with episodes of toxicity, according to 5-FU dose	
		370 mg/m^2/day (88 courses)	400 mg/m^2/day (110 courses)
Granulocytopenia (× 10^2 PMN/mm^3)			
15–20	3	5	1
10–15	4	2	2
< 10	6	1	6
		8	9
Thrombocytopenia (× 10^3 platelets/mm^3)			
100–150	1	1	1
50–100	2	1	1
< 50	1	1	0
		3	2
Oral mucositis*			
1	6	7	4
2	6	4	5
3	4	0	4
4	1	0	1
		11	14
Diarrhea*			
1	0	0	0
2	7	4	5
3	2	1	1
4	0	0	0
		5	6
Fever	6†	0	6
Alopecia	4‡		
Skin rash	2		
Neurologic	0		
Renal	0		

*Graded according to WHO recommendations (9).

†In 5 patients, the cause of fever was not documented microbiologically; in 1 it was due to *Staphylococcus aureus* septicemia.

‡Partial.

DISCUSSION

The action of 5-FU on cell metabolism occurs through two pathways: one involves the incorporation of 5-FU into RNA, and the other, conversion of 5-FU into fluorodeoxyuridylate (FdUMP), which inhibits TS and results in the suppression of DNA synthesis by depletion of thymidine triphosphate (10,11). FdUMP binds tightly to TS in the presence of the cofactor N_5-N_{10} methylene tetrahydrofolate (CH_2FH_4); this interaction leads to the formation of a slowly dissociating covalent ternary complex (TS-CH_2FH_4-FdUMP). In the absence of CH_2FH_4, binding of FdUMP to TS is noncovalent and weak (4, 12).

In folate-depleted L1210 leukemia cells, both the amount of ternary complex formed and the cytotoxicity of 5-fluorodeoxyuridine are reduced (4). In mouse Sar-

TABLE 4.—Mean PMN and platelet counts obtained at end of 21-day treatment intervals in 26 patients who received at least 4 courses*

Courses	Mean PMN count ($\times 10^2/mm^3$)	Mean platelet count ($\times 10^3/mm^3$)
1	51.9	341
2	48.1	355
3	40.2	311
≥ 4	45	305

*Variance analysis shows no significant difference either for PMN or for platelet counts obtained at the end of each time period in the 26 patients.

coma 180 and in human carcinoma Hep 2 cells, the cytotoxic activity of 5-FU was potentiated threefold when an excess of FA was added to the medium (5). Both experiments (4,5) showed that the folate requirement for optimal cell growth was much lower than that required for maximal cytotoxicity of fluoropyrimidines. In Friend leukemia cells, the growth-inhibiting effect of 5-FU was also increased by addition of FA; this potentiation did not occur in FA transport-defective cells (6). Moreover, in xenografts of human colorectal carcinoma cell lines resistant to fluoropyrimidines, the maximum binding of FdUMP to TS requires addition of exogenous folates (7).

For the above reasons, we assumed that in some tumors, the amounts of naturally occurring intracellular folates may be insufficient to allow optimal cytotoxicity of 5-FU. It has also been reported that if low doses of FA (3 mg) are administered in combination with 5-FU and semustine in patients with advanced colorectal carcinoma, the survival rate is not improved (13). We therefore thought that high doses of FA should effectively increase the cellular content of reduced folates and that they might overcome membrane transport deficiency for these compounds (6,14).

In previously untreated patients with colorectal cancer, we obtained a response rate (CR + PR) of 56%; in previously 5-FU-resistant patients, the response rate (CR + PR) was 21%. These objective response data suggest that simultaneous administration of 5-FU and high doses of FA can enhance the antitumor activity of 5-FU and that it also renders some colorectal tumors, previously resistant to 5-FU, sensitive to this drug. For gastric adenocarcinoma, the results were also encouraging in the small number of patients studied and during the short follow-up period; PR were recorded for three of five previously untreated patients.

In the present trial, toxicity was within acceptable limits. Side effects, when present, were prevented in subsequent courses by a 30-mg/m^2 decrease in the daily dose of 5-FU.

The combination of 5-FU with simultaneous high doses of FA should be tested in all tumors in which 5-FU has shown some therapeutic effectiveness. Biochemical and pharmacokinetic studies will be necessary for optimization of the doses and schedules of FA combined with 5-FU and other cytostatic fluoropyrimidines.

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Dianhydrogalactitol and Cisplatin in Combination for Advanced Cancer of the Uterine Cervix[1,2]

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Eighteen evaluable patients with squamous cancer of the uterine cervix recurrent after radiation therapy or with distant metastases were treated with dianhydrogalactitol (150 mg/m^2) and cisplatin (DDP) (50 mg/m^2), both given iv every 3 weeks. Only 39% of the patients responded, a result no better than that previously reported for DDP alone and only one-half as good as that previously reported by our group for a combination of this drug with mitomycin, vincristine, and bleomycin. Responses lasted a median of only 5 months. Hematologic toxicity was life-threatening in 22% of the patients and was severe in all but 11% of the remaining patients. We conclude that the addition of substantial and toxic doses of dianhydrogalactitol to DDP failed to substantially enhance the rate, quality, or duration of response compared to DDP alone. [Cancer Treat Rep 66:1809–1812, 1982]

Chemotherapy for advanced cancer of the uterine cervix is generally unsatisfactory. Cisplatin (DDP) is the single most active agent (1). In a study by the Gynecologic Oncology Group (GOG) using 50 mg/m^2 of DDP every 3 weeks, 11 of 22 patients without prior chemotherapy responded, while only two of 12 exposed to prior chemotherapeutic agents had objective responses (1). The median duration of response, measured from the start of remission to evidence of disease progression, was 6 months. Complete remission was noted in only three (14%) of the 22 patients given DDP as initial chemotherapy. Other agents, particularly doxorubicin and cyclophosphamide, have little activity alone or in combination (2).

In earlier pilot trials, we added DDP to combination programs of established efficacy: methotrexate plus bleomycin (3) and mitomycin plus vincristine plus bleomycin. Responses were frequent but short (4,5), and toxicity was severe with the former regimen.

In 1980, Blom et al (6) reported for the GOG that dianhydrogalactitol (DAG), an alkylating agent formulated for iv administration (7), was active against squamous cancers of the uterine cervix (6). In the GOG study, seven of 29 patients without prior chemotherapy (24%) responded to this drug; the responses included two complete remissions, one of which lasted > 22 months. The median duration of response was 4 months among all seven responding patients. No responses were noted among six other patients with prior chemotherapy. The dose of DAG in this trial was 60 mg/m^2 iv every 7 days.

The toxicity of DAG is predominantly hematologic, with infrequent and inconsistent reports of mild nausea and vomiting and occasional stomatitis. No end-organ toxicity other than myelosuppression has been reported (6,8–12). This makes DAG well-suited for combination with DDP, since the latter is only mildly myelosuppressive but can be nephrotoxic and produces moderately severe nausea and vomiting in most patients treated (1). The aims of the present study were to develop a safe combination chemotherapy schedule combining DAG and DDP and to make a preliminary estimate of the

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activity of the combination to determine if further investigations are indicated.

The choice of DAG dose was based on phase I trials (9) and on a phase II study in non-small cell lung cancer (11), where a dose of 150 mg/m^2 divided over 5 days every 4 weeks produced moderately severe hematologic toxicity with some frequency. In the treatment of glioblastoma multiforme, a single bolus dose of 150 mg/m^2 every 3 weeks had produced acceptable toxicity with the advantage of a more convenient schedule than daily administration for 5 days (12). A single dose of DAG every 3 weeks was chosen for the present combination trial, to be given with 50 mg/m^2 of DDP (the effective dose in the GOG study), to allow for an accurate definition of nadir blood cell counts in each cycle as a basis for later dose modification. This was believed to be safer than the administration of small weekly doses, since the weekly schedule would allow the administration of further DAG at a time when toxic doses had already been administered but hematologic toxicity was not yet fully apparent.

PATIENTS AND METHODS

Eligibility was restricted to women with squamous or adenosquamous cancer of the uterine cervix with disease that could be measured in two diameters (or at least evaluated for response to chemotherapy), serum creatinine $\leq$ 1.5 mg/dl, wbc count $\geq$ 4000/μl, platelet count $\geq$ 100,000/μl, no prior chemotherapy with any protocol drugs, and tumor beyond hope of cure by surgical excision or radiation therapy. The patient was required to give informed consent prior to entry in the study.

Protocol drug administration included 50 mg/m^2 of DDP and 150 mg/m^2 of DAG iv every 3 weeks. DDP was administered 30 minutes after 40 mg of iv furosemide and the beginning of a 2-hour infusion of 2 L of 5% dextrose in 0.45% NaCl containing 10 mEq of KCl/L. Mannitol (12.5 g) was given just prior to the DDP. This regimen of DDP administration has proved safe and effective for outpatient use (13). DAG was administered over 1 hour in 1 L of fluid, usually the second liter of iv 5% dextrose in 0.45% saline stipulated in the protocol for DDP administration.

Doses were reduced to 67% for wbc counts of 3000–4000/μl or platelet counts of 75,000–100,000/μl. Chemotherapy was withheld on a week-by-week basis if either blood cell count was below the stipulated level. Blood cell counts were routinely obtained on the days of planned chemotherapy and 2 weeks thereafter. Any wbc count $<$ 1500/μl, granulocyte count $<$ 750/μl, or platelet count $<$ 40,000/μl led to a permanent reduction of DAG dose to 100 mg/m^2, which could be further reduced if blood cell counts on the day of planned chemotherapy were depressed.

Complete remission was defined as the complete disappearance of all signs and symptoms of disease. Partial response was defined as a $\geq$ 50% decrease in the sum of the products of perpendicular cross-sectional diameters of measurable lesions. Disease progression was defined as an increase of $\geq$ 25% in the sum of the products of perpendicular diameters of measurable or evaluable lesions, the appearance of new lesions, a $>$ 10% weight loss, or a decrease in performance status $>$ 1 point on the Eastern Cooperative Oncology Group (ECOG) scale (0 = fully functional; 1 = ambulatory with symptoms; 2 = bedridden less than one-half of the day; 3 = bedridden more than one-half of the day; and 4 = fully bedridden).

Treatment was continued until disease progression was noted. A minimum of two cycles of chemotherapy were planned for each patient.

Twenty-three patients were entered in the trial between November 1979 and March 1981. Five patients have been excluded from the present analysis. One patient has no follow-up available, and two started chemotherapy with markedly reduced doses of one or both agents and were never given full doses. Neither of these patients responded, but one did have life-threatening hematologic toxic effects. Since response might have

TABLE 1.—Patient characteristics

	All patients (18)	Responders (7)	Nonresponders (11)
Prior chemotherapy	6%	0%	9%
Performance status 0 or 1	78%	71%	82%
Weight loss > 5% in 6 mos	39%	45%	29%
Age in yrs			
Median	52	51	53
Range	40–65	46–65	40–65
Mos from diagnosis to entry			
Median	11.5	11	12
Range	0–121	0–118	6–121

TABLE 2.—Response according to site of involvement

Site	No. of patients—	
	Responding	With involvement
Pelvis and other sites	1	6
Pelvis only	2	5
Abdominal wall	2	3
Lung	2	3
Para-aortic nodes (by computerized tomography)	1	6

occurred at full doses in one or both of these patients, they are excluded from further analysis. The other two patients who are excluded from analysis died 2 weeks after the first dose of chemotherapy, presumably of disease, but without further follow-up information available. The inclusion of any or all of these five patients in the present analysis would not alter the conclusions reached. Their exclusion, however, allows a more clear-cut definition of toxicity and efficacy.

Some of the patient characteristics are summarized in table 1. Only one patient was bedridden more than one-half the day, and only five patients had lost > 10% of their body weight in the previous 6 months. All patients had squamous cancer of the uterine cervix with a single exception, an adenosquamous cancer in a patient who did achieve partial remission. The pathologist at the time of diagnosis called tumor well-differentiated in three patients, moderately differentiated in six, and poorly differentiated in five. Differentiation was unknown in four patients. The interval from diagnosis to entry was between 0 and 13 months in 11 patients, 27 and 49 months in four patients, and 116 and 121 months in three patients.

One patient who had lung metastases at presentation had received no prior anticancer therapy, 12 had had prior radiation therapy alone, four had had prior major surgical procedures as well as irradiation, and one had had radiation therapy and single small doses of methotrexate and bleomycin.

Sites of involvement followed for response are given in table 2. More than one site was followed in six patients.

RESULTS

Among the 23 patients entered, 18 of whom were considered fully evaluable for response and toxicity, two achieved complete remission for 5 $^1/_2$ and 21+ months, and five achieved partial remissions for 2+, 3, 4, 5, and 6 months.

One nonresponding patient received only one dose of chemotherapy before she refused further therapy (her abdominal computerized tomogram was unchanged at this point). The other ten nonresponding patients received two doses (four patients), three doses (one), four doses (one), five doses (three), and nine doses (one) of DAG-DDP before disease progression was apparent. Table 1 fails to reveal patient characteristics predictive of response to DAG-DDP. Four of 11 patients with intervals from diagnosis to start of chemotherapy between 0 and 13 months responded, as did one of four with intervals of 27–49 months and two of three with intervals of 116-121 months. The one patient without prior radiation therapy responded, as did two of four with prior surgery plus radiation therapy. As shown in table 2, responses were seen at least once in each site that was evaluated for response.

Median survival is 9 months among the 18 evaluable patients. Four of seven responders have died. The median survival of the responders is projected at 15 months and will not be < 10.5 months. Two responders are alive (one with obvious disease) at 21 and 24 months from entry. Median survival of the 11 nonresponders, eight of whom have already died, is 8 months, with the longest survival being 12 months from entry. Responders did not live significantly longer than nonresponders (log-rank test).

Toxicity

As anticipated from the dose of DAG chosen, the major toxicity was hematologic. Fourteen patients (78%) required packed rbc transfusions. Three (17%) had platelet counts < 25,000/μl at some point, with major hemorrhage from vagina and rectum in one patient each. Four more patients (22%) had nadir platelet counts of 25,000–49,999/μl, and five others (28%), 50,000–74,999/μl. Nadir wbc counts < 1000/μl were seen in two patients (11%); 1000–1900/μl, in seven (39%); and 2000–2999/μl, in seven (39%). Two patients had increases of the serum creatinine to > 1.5 mg/dl; one patient had a peak level of 1.7 mg/dl, and another had a peak level of 1.6 mg/dl, which first rose only several weeks after DDP administration but returned to the pretreatment level of 1.3 mg/dl promptly on rehydration. Thus, there was only one instance of mild azotemia from DDP. One patient had repeated anaphylactic shock from DDP in spite of steroid and antihistamine pretreatment, which led to cessation of therapy 16 months after having achieved a complete remission. One patient developed a perforated duodenal ulcer while in remission 2 days after her second dose of chemotherapy, and survived surgical plication. The relation of this event to the chemotherapy is uncertain.

DISCUSSION

The results observed with a combination of DAG and DDP are not substantially better than those previously reported for DDP alone, although the number of pa-

tients given the combination was small. Among patients without prior chemotherapy, moderate doses of DDP alone yielded responses in 50% of patients treated, with a median response duration of 6 months and a median survival of 9 months among responding patients (1). While the response rate has been only 25% in a subsequent GOG trial of different doses of DDP in cervical cancer (14), the response rate of 39% observed in the present trial remains in the range of that seen with DDP alone.

The very high degree of hematologic toxicity observed with DAG-DDP argues that the DAG dose was sufficiently high, since this dose of DDP is only mildly myelosuppressive (1). Perhaps more activity would have been observed if DAG had been administered weekly, as it had been in the original positive phase II trial (6). Some schedule specificity has been observed for DAG in animal systems, but none has been demonstrated in man (8). In tissue culture, DAG cytotoxicity is independent of the phase of the cell cycle (7). Moderate antitumor efficacy (15% responses) in human squamous cancer of the lung has been demonstrated for DAG at a dose of 30 mg/m^2 iv daily for 5 days every 4–5 weeks.

The results of the present trial are particularly disappointing when compared to the previous results observed with DDP combined with mitomycin, vincristine, and bleomycin (BOMP). Responses with both regimens were short (median of 4 months for BOMP and 5 months for DAG-DDP), but the response rate was twice as high for BOMP as for DAG-DDP (78% vs 39%) (5). The 18 evaluable patients in the DAG-DDP study were quite similar in clinical characteristics to the 13 evaluable patients in the BOMP study, so that the apparent superiority of BOMP is unlikely to relate to patient selection.

We conclude that the addition of DAG to DDP in the chemotherapy for advanced cancer of the uterine cervix did not substantially improve response rate or response duration, while it did produce substantial hematologic toxicity. Major resources should not be expended conducting phase III comparative studies of DAG-containing combination chemotherapy programs in cervical cancer unless other pilot studies provide a great deal more favorable evidence of its activity in combination than was found in the present trial.

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Phase I–II Study of *m*-AMSA Administered as a Continuous Infusion[1]

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In vitro studies of 4′(9-acridinylamino)-methanesulfon-*m*-anisidide (*m*-AMSA) with L1210 leukemia cells have demonstrated that a given level of DNA breakage and intracellular *m*-AMSA concentration depends upon the presence of a continuous concentration of extracellular drug and that cytotoxicity increases with an increasing duration of drug exposure. Since previous studies had shown that free *m*-AMSA has a half-life of 6–8 hours, a trial of *m*-AMSA given as a continuous infusion was undertaken in patients with solid tumors and lymphomas. Patients were treated with 30 mg/m²/day × 3, 30 mg/m²/day × 4, or 40 mg/m²/day × 3. Myelosuppression was dose-limiting and occurred at about Day 13 after the start of the infusion. Recovery was noted by Day 21. There was no evidence of cumulative hematologic toxicity. The maximally tolerated dose was 30 mg/m²/day × 3 in our patient population. Mild phlebitis occurred in all patients. There were no instances of gastrointestinal, hepatic, renal, cardiac, or neurologic toxic reactions. Evidence of some antitumor effect was seen in three patients. We conclude that it is possible to give *m*-AMSA as a continuous infusion and that studies should be undertaken in patients with acute myelogenous leukemias to compare the efficacy of *m*-AMSA infusion with conventional bolus administration as a means to enhance the duration of response. [Cancer Treat Rep 66:1813–1817, 1982]

4′-(9-Acridinylamino)-methanesulfon-*m*-anisidide (*m*-AMSA) has been shown to be a promising agent in the treatment of anthracycline-resistant myelogenous leukemias (1,2). In the treatment of solid tumors, the commonly employed means of drug administration is either a single large bolus iv (120 mg/m² every 3–4 weeks) or smaller daily boluses iv (30–40 mg/m²/day for 3 days every 3–4 weeks) (3,4). The dose-limiting toxic effect of these two schedules has been myelosuppression, occurring about 10–14 days after drug administration, with recovery by Day 21. Much higher doses have been used in the treatment of acute leukemias, and with higher doses, mucositis and gastrointestinal toxicity have been significant problems (1,5). Cardiac and neurologic toxic effects have been reported, although the exact incidence of either of these two complications is unknown (6,7).

In man, *m*-AMSA exists in the serum as both free and bound parent drug and metabolites. The principal route of excretion appears to be the biliary system (8,9). Previous pharmacokinetic studies have shown that free *m*-AMSA is cleared from the serum with an initial half-life of 11–15 minutes and a slower terminal half-life of 6–8 hours (9,10). By 24 hours after a single iv bolus, the free drug has decreased by a factor of 100 (11). What is not known, however, is the optimal concentration of *m*-AMSA and duration of exposure for the drug to be cytotoxic to human malignant cells.

Recent work has shown that DNA single-strand breaks produced by *m*-AMSA in L1210 cells disappeared within 20 minutes following removal of the drug (12). Second, drug egress from the cells in transport studies was very rapid following removal of the drug from the extracellular medium (12). Finally, soft agar colony survival studies using L1210 cells have suggested a schedule dependency; ie, a low concentration of drug for long exposure times was more cytotoxic than a much higher concentration of drug for short exposure times (L. A. Zwelling, D. Kerrigan, and S. Michaels, manuscript submitted).

Thus, since a given level of DNA breakage and intracellular *m*-AMSA concentration depended upon the

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presence of a continuous concentration of extracellular *m*-AMSA, since cytotoxicity increased with increasing duration of drug exposure, and since the half-life of the free drug is short, we proposed a trial of *m*-AMSA administered as a continuous infusion.

MATERIALS AND METHODS

Clinical Study

Twenty-two patients were entered in the study over a 6-month period. Patient characteristics are shown in table 1. Fourteen females and eight males received 31 courses of the drug. All courses were evaluable for toxicity, and 20 of the 22 patients were evaluable for response. The median age was 50 years and the median performance status was 70%. Lymphoma, breast cancer, and ovarian cancer were the most frequent diagnoses in the study population. All patients except two had been pretreated with drug regimens containing at least four myelosuppressive agents, and 14 of the 22 patients had received doxorubicin either as a single agent or in combination prior to receiving *m*-AMSA. Nine of the 22 patients had also been previously treated with radiotherapy.

TABLE 1.—Characteristics of patients treated with *m*-AMSA

Characteristic	No. of patients
Total in study	22
Females	14
Males	8
Median age in yrs (range): 50 (23–67)	
Median Karnofsky performance status: 70%	
Diagnosis	
Lymphoma	7
Breast cancer	5
Ovarian cancer	5
Sarcoma	2
Melanoma	2
Testicular cancer	1
Previous treatment	
Radiotherapy + chemotherapy	9
Chemotherapy alone	13

Prior to entry in the study, each patient had a history and physical examination, ECG, cbc with differential and platelet counts, routine automated chemistries, and appropriate baseline radionuclide scans and X-rays. To be eligible for study, the patients must have had evaluable disease, a life expectancy of at least 1 month, normal renal and hepatic function tests, a wbc count $\geq$ 3500/μl, and a platelet count $\geq$ 100,000/μl. Exceptions were made in any of the patients with abnormal pretreatment hemograms or liver function tests if the abnormalities were thought to be due to malignancy. Informed consent was obtained in writing from all patients.

All treatments were given on an inpatient basis. Initial patients entered in the study were treated with 30 mg/m^2/day for 3 days. If no toxic reaction was seen, subsequent patients were treated with 30 mg/m^2/day for 4 days. The intent was to increase the infusion by 24 hours with every three patients until the dose-limiting toxicity was reached. Further patients would then be given 40 mg/m^2/day for 3 days, with escalations as above until the dose-limiting toxicity was reached. Thus, it would be possible to determine a maximally tolerated dose and infusion duration for patients treated with either 30 or 40 mg/m^2/day.

Solutions of *m*-AMSA were prepared in the pharmacy. The appropriate amount of *m*-AMSA in a vehicle of L-lactic acid and *N*, *N'*-dimethylacetamide was added to 1000 ml of 5% dextrose in water (D5W), to which 1 mEq of sodium bicarbonate had been added. The resultant solution was infused via a peripheral vein utilizing an IVAC pump (IVAC Corp, San Diego). Each liter of solution was administered over a 12-hour period. One patient with only one arm and two other patients who had no peripheral venous access site had the drug administered via a central vein.

Four patients who had either previously received 550 mg/m^2 of doxorubicin or who had cardiac symptoms underwent continuous ECG monitoring during the infusion of *m*-AMSA.

After the infusion, patients were discharged and interim blood cell counts and liver function tests were obtained twice weekly. Re-treatment was expected every 21–28 days or when peripheral blood cell counts had recovered. After two cycles of drug treatment, patients were evaluated as to disease status. If disease progression had occurred, treatment was discontinued. Patients were evaluated according to standard response criteria defined as follows: *complete remission*—the disappearance of all evaluable disease; *partial remission*—a $>$ 50% decrease in the product of the two longest perpendicular diameters, lasting 4 weeks; *disease stabilization*—a $<$ 50% decrease in the product of the two longest perpendicular diameters, lasting 4 weeks; and *disease progression*—the appearance of new lesions or a $>$ 25% increase in the product of the two longest perpendicular diameters. To be evaluable for response, patients had to have received at least one course of drug and been examined at the National Cancer Institute to determine disease status prior to death.

Pharmacokinetics

Blood samples were obtained by venipuncture at 12–24-hour intervals following the start of the infusion. A 2-ml aliquot of serum was added to 10 ml of ethyl acetate and the mixture was shaken vigorously. Under these

conditions nearly 100% of free *m*-AMSA will extract into the ethyl acetate. The ethyl acetate (5 ml) was separated from the aqueous phase and stored at −20°C until further analysis (10). Preparation for high-performance liquid chromatography analysis consisted of evaporating the 5 ml of ethyl acetate to dryness and resuspending the residue in 2 ml of 0.01 M phosphate buffer (pH 8). The sample was adjusted to pH 2 by the addition of 0.5 ml of HCl (0.5 N). The solution was extracted with 7 ml of *n*-hexane and the hexane was then discarded. A solution of saturated sodium borate (3 ml) was added to the remaining aqueous phase, and the solution was extracted twice with 5 ml of ethyl acetate. The ethyl acetate fractions were pooled and evaporated to dryness. The residue was dissolved in 200 μl of a solution of methanol:water (9:1). A 100-μl sample was injected onto an Altex high-performance liquid chromatography system, using a 10-μl Spherisorb ODS 4.2 × 25-cm column. The mobile phase was methanol:0.004 M sodium heptanesulphonate (4:1) pumped at 2 ml/minute, and the column was monitored at 254 nm with a Gilson model 111 LC detector (L. Malspeis, unpublished method).

RESULTS

Pharmacokinetics

Free *m*-AMSA plateaued in the serum of patients by 24 hours and remained constant until the end of the infusion at 72 or 96 hours. The mean steady-state concentration of free *m*-AMSA in eight patients receiving 30 mg/m²/day for 3 or 4 days was 0.15 ± 0.097 μM. Two patients who received 40 mg/m²/day for 3 days had steady-state *m*-AMSA values of 0.11 and 0.25 μM, respectively.

Toxicity

The toxic reactions observed were mainly myelosuppression and phlebitis.

Myelosuppression

Blood cell count suppression as a function of drug dose is shown in table 2. At a dose of 30 mg/m²/day for 3 days, 12 patients received 18 evaluable cycles of the drug: seven received one cycle, four received two cycles, and one received three cycles. The median time to retreatment was 34 days from the start of the *m*-AMSA infusion.

Eight drug cycles resulted in nadir wbc counts < 2000/μl and two in nadir wbc counts < 1000/μl. The median nadir wbc count was 2100/μl, occurring on about Day 13 with recovery by Day 20. The two patients who had nadir wbc counts < 1000/μl had breast cancer, a poor performance status, and a history of extensive irradiation. Two patients had febrile episodes during treatment and both recovered with broad-spectrum antibiotic administration.

Eight cycles produced a nadir platelet count < 50,000/μl. The median nadir platelet count was 36,000/μl, occurring on Day 15 with recovery by Day 21. One patient had a nadir platelet count < 20,000/μl but this was associated with advancing infiltrative marrow disease. There were no hemorrhagic episodes and no deaths attributable to drug toxicity in this group. In the five patients who received more than one cycle of drug, there was no evidence of a cumulative hematologic toxicity.

Seven patients received ten evaluable cycles of drug at a dose of 30 mg/m²/day for 4 days: five patients re-

TABLE 2.—Hematologic toxicity of *m*-AMSA administered as a continuous infusion

	30 mg/m²/day × 3	30 mg/m²/day × 4	40 mg/m²/day × 3
No. of patients	12	7	3
No. of cycles	18	10	3
No. of cycles with wbc count nadir < 2000/μl	8	6	3
No. of cycles with wbc count nadir < 1000/μl	2	5	3
Median wbc count nadir (range)/μl	2100 (500–7600)	1600 (600–4000)	200 (100–900)
Median day of wbc count nadir (range)*	13 (8–18)	15 (9–16)	13 (12–13)
No. of cycles with platelet count nadir < 100,000/μl	8	7	3
Median platelet count nadir (range)/μl	36,000 (11,000–525,000)	38,000 (3000–212,000)	109,000;49,000;7000 (individual values listed)
No. of febrile episodes	2	2 (1 septic death)	2 (1 septic death)

*All days are counted from start of *m*-AMSA infusion.

ceived one cycle, one received two cycles, and one received three cycles. Six cycles produced a nadir wbc count $< 2000/\mu l$, with five producing a nadir wbc count $< 1000/\mu l$. The median nadir wbc count was $1600/\mu l$, occurring at about Day 15 with recovery by Day 22. The median nadir platelet count was $38,000/\mu l$, occurring about Day 17. In this treatment group there were two febrile episodes, with one death due to sepsis. One patient required platelet transfusions for counts $< 20,000/\mu l$. There was no evidence of cumulative hematologic toxicity in the two patients who received multiple courses of the drug.

Only three patients were treated at a dose of 40 mg/m^2/day for 3 days and all three had severe myelosuppression (table 2). There were two febrile episodes in this group, with one death due to *Listeria* meningitis. Two of the three patients had breast cancer and a poor performance status and had received extensive treatment prior to receiving *m*-AMSA; one of these two had received lumbosacral irradiation concomitantly with *m*-AMSA treatment.

Phlebitis

All patients experienced phlebitis at the injection site. Initially, the daily dose of drug was administered in a total volume of 1000 ml of D5W over 24 hours. Under these conditions, the phlebitis was severe, causing significant discomfort by about 6 hours and usually necessitating a change in the iv site by 12 hours. Administering each day's dose of drug in a total volume of 2000 ml of D5W, with 1 mEq of sodium bicarbonate/L of solution, significantly lessened the phlebitis. Under these conditions of administration, phlebitis occurred by 24 hours and the iv site had to be changed every 24–36 hours. The phlebitis was characterized as tenderness and redness without warmth at the injection site and usually extended several inches beyond the iv site. The discomfort was tolerable; no infusion had to be stopped and no patient refused further therapy because of pain. The soreness in the arm resolved in about 3 days. Three drug extravasations occurred. There was no necrosis and only minor soreness for several days. Two of the patients were receiving corticosteroids for central nervous system metastases and both of these patients had minimal problems with phlebitis.

Other Toxicity

No patients had nausea or vomiting during the infusion. One patient developed mild mucositis on Day 7.

One patient with known liver involvement from embryonal cell carcinoma of the testes developed a transient rise of SGOT and SGPT, which peaked at Day 17 and became normal by Day 34. Seven patients developed elevations of alkaline phosphatase while receiving therapy, but all seven had known metastatic disease in the liver and the majority of them had evidence of disease progression in the liver as the alkaline phosphatase level rose.

None of the patients had ECG changes before, during, or after the *m*-AMSA infusion. Three of four patients who underwent constant ECG monitoring during the infusion showed no evidence of arrhythmia. The fourth patient, who had a history of Wolff-Parkinson-White syndrome and was receiving therapy with quinidine sulfate, had one episode of supraventricular tachycardia while receiving *m*-AMSA, but this terminated spontaneously. He had had occasional attacks of palpitations prior to receiving *m*-AMSA, however, despite being on antiarrhythmic therapy.

Responses

Evidence of some antitumor effect was seen in three patients. One patient with nodular poorly differentiated lymphoma had resolution of generalized lymphadenopathy by Day 4 of the infusion (30 mg/m^2/day for 4 days). This patient, who had received total-body irradiation 7 years previously, was unable to be re-treated because of persistent severe thrombocytopenia. The lymphadenopathy subsequently returned by Day 21.

The other two effects were seen in patients with ovarian cancer. One patient had a large pelvic mass with hydronephrosis and while receiving *m*-AMSA had a $< 50\%$ shrinkage of the mass, with improvement of the hydronephrosis lasting > 24 weeks. The other patient with ovarian cancer had disease stabilization for 16 weeks.

DISCUSSION

Two questions need to be answered when the schedule of drug administration is changed: (*a*) is the toxicity schedule-dependent and is the pattern of toxicity altered? and (*b*) is it efficacious to give the drug in this fashion?

We have demonstrated that the administration of *m*-AMSA as a continuous infusion is feasible. When administered via a peripheral vein, a mild to moderate phlebitis ensued which resolved within 3 days and was reasonably well-tolerated. Furthermore, except for the phlebitis, the pattern of toxicity was not altered by administering *m*-AMSA as a continuous infusion. Hematologic toxicity was dose-limiting and reversible. Thrombocytopenia was seen frequently. Published studies using *m*-AMSA as a single iv daily bolus have noted only mild thrombocytopenia when the platelets were affected (3,10,13). Thus, it is possible that the thrombocytopenia seen in our study might represent a schedule-dependent toxicity. However, three recent studies in patients with lymphoma, prostate cancer, and small cell lung cancer have noted a higher incidence of

thrombocytopenia than that reported in other studies (14–16). These three studies suggest that the degree of thrombocytopenia in our data is not a reflection of schedule-dependent toxicity but rather reflects the pretreatment and poor bone marrow reserve of our patient population.

The maximally tolerated dose of *m*-AMSA in this study was 30 mg/m^2/day for 3 days. This dose produced wbc count nadirs < 2000/μl and platelet count nadirs < 50,000/μl in approximately 50% of the patients. We recommend this as the initial starting dose in adult patients with solid tumors who have poor performance status or poor bone marrow reserve or are receiving concomitant radiotherapy to large marrow-containing areas. Because of the extensive prior treatment received by our patients, our study cannot resolve the issue of the maximally tolerated dose of *m*-AMSA in patients who have good bone marrow reserve.

While we have demonstrated the feasibility of giving *m*-AMSA as a continuous infusion, the question of efficacy cannot yet be answered. Efficacy is the sum total of response rate balanced against overall toxicity. To have increased efficacy, a change in the drug schedule should demonstrate either (*a*) an increased response rate with no change or a decrease in toxicity or (*b*) no change in the response rate with a decrease in toxicity when compared to other schedules.

Recent studies have shown that when *m*-AMSA is given as a bolus to patients with solid tumors, the reponse rate is virtually zero. In fact, the clinical spectrum of activity of *m*-AMSA as a single agent is remarkably narrow, being relegated to treatment of the lymphomas and the myelogenous leukemias. We feel that in order to test efficacy, the schedule change we have studied for *m*-AMSA needs to be studied systematically in the myelogenous leukemias and the lymphomas, both tumors with known significant response rates to *m*-AMSA (1,2,5,14).

In the myelogenous leukemias, large bolus doses are used and have been associated with significant gastrointestinal toxicity (5). Continuous infusion might avoid the gastrointestinal toxicity. Our data demonstrated that steady-state values of free *m*-AMSA are reached at the end of the first day of infusion. More importantly, peak or steady-state levels achieved by continuous infusion are 1%–2% of those peak serum levels attained with bolus administration of the drug (10). Recently, the cardiac toxicity of doxorubicin has been found to be reduced by administering the anthracycline as a continuous infusion (17,18). Thus, if acute cardiac toxicity of *m*-AMSA is related to high peak serum concentrations of drug, then continuous infusion of AMSA might avoid this dramatic and sometimes lethal side effect. Finally, if concentration-times-time effects are important in killing human leukemia cells and if cytotoxicity of *m*-AMSA is schedule-dependent in man, then the continuous infusion of *m*-AMSA might improve the response rate and duration of responses in the leukemic population.

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Pharmacokinetic Studies of 4′-Epi-doxorubicin in Cancer Patients With Normal and Impaired Renal Function and With Hepatic Metastases[1]

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The plasma pharmacokinetics of 4′-epi-doxorubicin (4′-epiDX), a new anthracycline derivative, was investigated in 22 cancer patients after single iv bolus administration. Eleven patients had normal kidney and liver functions, five had impaired renal function, and six had liver metastasis. Plasma levels (up to 96 hours) of the unaltered drug and of one metabolite (4′-epi-doxorubicinol) were specifically determined by a highly sensitive high-pressure liquid chromatography method with fluorescence detection (in 19 patients after the first course and in five patients after the fourth course). A triexponential decrease in the plasma levels of the unaltered drug, with a long terminal plasma half-life, was observed in all patients. The metabolite levels were always lower than those of the parent compound. The main pharmacokinetic parameters of 4′-epiDX in patients with normal kidney and liver functions were (mean ± SE): half-life = 40 ± 6 hrs, plasma clearance = 0.882 ± 0.075 L/min, and volume of distribution = 46.1 ± 7.5 L/kg, suggesting that the extensive extravascular distribution is the main determinant of the slow elimination of the drug. No significant differences between normal and impaired renal function were found with respect to the pharmacokinetics of 4′-epiDX. In patients with liver metastasis, changes in the disposition of the drug were observed, which involved the area under the curve and plasma clearance. The observations suggest that in cancer patients with liver metastasis, dose modification of 4′-epiDX is required. [Cancer Treat Rep 66:1819–1824, 1982]

4′-Epi-doxorubicin (4′-epiDX) is a new anthracycline derivative closely related to doxorubicin (DX), with a broad spectrum of activity against animal tumors (1,2). Preclinical studies indicate less systemic and cardiac toxicity than that of DX at similar equipotent therapeutic doses (2).

4′-EpiDX is currently under extensive phase II clinical investigation. The results obtained so far suggest that the drug is as effective as DX against a broad spectrum of human neoplasms and better tolerated in terms of conventional toxicity (3–6). Specific studies of chronic cardiac toxicity are in progress. Experimental studies in animals (2,7), as well as a preliminary crossover comparative pharmacokinetic study in a group of cancer patients after single iv administration of 60–75 mg/m^2 of DX and 4′-epiDX (8), show a more rapid disappearance of 4′-epiDX in comparison with DX.

At present, no definite data are available as to the fate of this drug after administration in man, with particular reference to the metabolic pathway and routes of excretion. However, preliminary data (unpublished observations) indicate that urinary excretion of 4′-epiDX, as well as one of its metabolites, the 13-dihydro derivative 4′-epi-doxorubicinol (4′-epiDXol), accounts for only about 10% of the administered dose, while very high concentrations of both compounds are found in bile. It appears, therefore, that the fate of 4′-epiDX is qualitatively similar to that of DX.

The aim of the present study was to investigate some clinical pharmacokinetic aspects of 4′-epiDX with particular reference to interpatient variation in drug clearance and the possible pharmacokinetic consequences of renal impairment and liver metastasis.

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MATERIALS AND METHODS

Patients

Twenty-two patients with solid tumors who were admitted to the Oncology Division of Ospedale M. Malpighi, Bologna, Italy, were studied. All patients had received prior chemotherapy and/or irradiation or hormonotherapy. These oncologic treatments were discontinued at least 1 month before, and any other treatment 2 days before, the start of the study.

Eleven patients had normal hepatic and renal functions, five had impaired kidney functions (elevated serum creatinine and BUN), and six had liver metastasis and showed abnormalities in serum liver function tests (bilirubin, SGOT, SGPT, and alkaline phosphatase).

Chemicals

4′-EpiDX·HCl for clinical use and the pure analytic standards of 4′-epiDX, 4′-epiDXol, and daunorubicin were supplied by Farmitalia Carlo-Erba (Milan, Italy). Other chemicals were supplied by Merck (Darmstadt) or Farmitalia Carlo-Erba (Milan) and were of analytic grade.

Drug Administration

4′-EpiDX was administered as a rapid iv infusion over a 1–2-min period. The dose administered ranged from 35.7 to 90 mg/m^2. As a rule, the highest dose was given to patients with normal hepatic and renal functions.

Blood Samples

Blood samples (4 ml) were drawn at 0, 15, 30, and 60 mins and at 2, 3, 4, 6, 8, 10, 24, 48, 72, and 96 hrs after drug administration. Samples were immediately centrifuged and the separated plasma was kept frozen at −20°C until analysis.

Drug Analysis

4′-EpiDX and 4′-epiDXol in plasma were specifically determined by a highly sensitive high-pressure liquid chromatography method.

Extraction Procedure

To the plasma samples in silanized centrifuge tubes were added 100 μl of a stock solution of daunorubicin hydrochloride (as internal standard; about 1 μl/ml), 1 ml of phosphate buffer, and 10 ml of chloroform:heptanol (9:1). The mixture was shaken for 20 mins (horizontal shaker, 120 impulses/min) and then centrifuged at 4000 rpm for 10 mins. The organic layer was collected and extracted with 0.5 ml of 0.3 M phosphoric acid in a vortex mixer. The acidic phase, after treatment with 2 ml of *n*-hexane to remove nonpolar contaminants, was analyzed by high-pressure liquid chromatography (10–100-μl injections).

Chromatographic Analysis

Chromatographic analysis was performed with a Perkin Elmer series 3B liquid chromatograph equipped with a 650-10 LC fluorescent detector (470-nm excitation wavelength, slit width 10 nm; 580-nm emission wavelength, slit width 20 nm). In addition, a Supelcosil LC-18 column (15 cm × 4.6 mm internal diameter) was used, and quantitation was performed with a Perkin Elmer Sigma-10 data system. The mobile phase consisted of methanol:water (3:1) plus 0.5% acetic acid and 2.5 mM sodium pentane sulphonate. Several authors reported considerable separation for DX and daunorubicin when using reversed-phase columns and methanol or acetonitrile and phosphate buffer as the mobile phase. We found that the use of paired ion chromatography improves the peak shapes and reduces the analysis time. The method was calibrated in the usual way, with standards made by supplementing blood bank plasma with stock solutions of the drug. The response was linear in the experimentally observed range of concentrations. The coefficient of variation on replicate analyses was 8% for plasma levels ⩾ 3 ng/ml.

Pharmacokinetic Analysis

Visual inspection of the plasma-level profile of unchanged 4′-epiDX shows that a triexponential curve adequately describes the pattern of plasma-level decreases according to the equation

$$Cp\,(t) = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} + C \cdot e^{-\gamma t}.$$

The values of the parameters of the triexponential function were determined by submitting the individual plasma-level curves to a nonlinear least-squares fit computer program (MINUIT, CERN Library) and a multistage program using both SIMPLEX (9) and a Davidson and Fletcher algorithm (10). Each minimization process was followed by an extensive search of other possible local minima and the analysis of the "error hole" map (11).

Calculated curves fit closely to experimental values within the time interval investigated (15 mins to 96 hrs); for some patients, abnormally high plasma levels were extrapolated in the initial interval (0–15 mins). As a consequence of these extrapolation problems, a reliable estimation of some parameters in terms of the conventional three-compartment open model cannot be safely made. Therefore, only the slow disposition rate with the corresponding biologic terminal half-life, the area

under the plasma-level curve (AUC), the plasma clearance (Pl Cl = dose/AUC), and the apparent volume of distribution at pseudoequilibrium (Vd = Pl Cl/γ) were taken into account in this discussion. The AUC (0–∞) was estimated by adding AUC (15 mins to ∞, calculated from the computer-fitted curve) to the rectangular area in the interval 0–15 mins (with height equal to the plasma level measured at 15 mins).

To compare the pharmacokinetic properties of the drug in different patients, AUC was linearly corrected for each dose administered. For the metabolite 4′-epiDXol, the pharmacokinetic parameters taken into account were the AUC (calculated by trapezoidal rule) up to 96 hrs and the ratio between the AUC of the metabolite and the AUC of the unaltered drug.

RESULTS

The time course of 4′-epiDX and 4′-epiDXol plasma levels after iv administration of 90 mg/m² to patients with normal renal and hepatic functions is shown in figure 1 (semilog plot, mean values of nine patients). The plasma-level profile of 4′-epiDX appears to be triexponential, with initially very rapid decrease (lasting 1–2 hrs) and a slow terminal linear phase (established 24 hrs after drug administration). Mean plasma levels of 4′-epiDXol were substantially lower than those of the parent compound over the whole time interval investigated. The plasma-level curves of the two compounds were nearly parallel during the terminal linear phase.

The individual values of the 4′-epiDX kinetic parameters in patients with normal hepatic and renal functions are listed in table 1. The mean terminal plasma half-life was about 40 hrs (coefficient of variation [CV] = 49%), indicating a slow elimination of the drug, in spite of its very efficient mean Pl Cl of about 0.88 L/min (CV = 28%). The apparent discrepancy between the slow elimination and the very efficient clearance process can be explained by an extensive partition of the drug in the tissues, suggested by the very high apparent Vd (46 L/kg, CV = 54%). As regards 4′-epiDXol, its AUC was about 25% (CV = 39%) of that of its parent compound.

The parallelism between the plasma-level curves of the two compounds during the linear terminal phase suggests that the rate constant for metabolite elimination is at least as large as that of the unaltered drug (12). No substantial differences were detected between the first and the fourth treatment courses. In the two patients who were studied on both occasions, the pa-

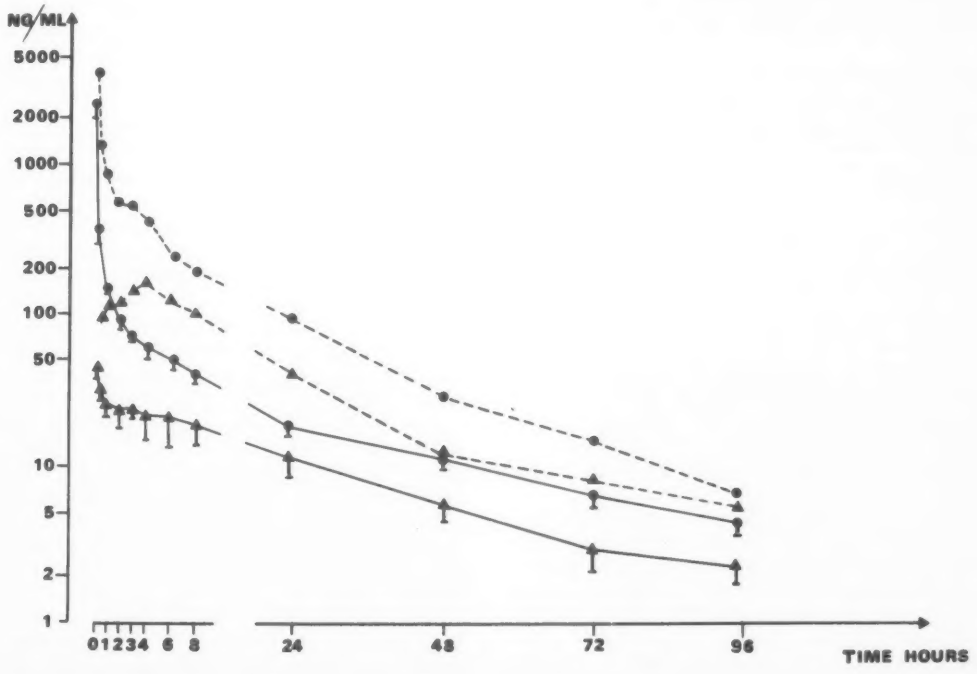

FIGURE 1.—4′-EpiDX (●) and metabolite 4′-epiDXol (▲) concentrations following 90 mg/m² iv bolus doses in 9 patients with normal hepatic and renal functions (solid line; mean ± SE) and in patient 17 with hepatic metastases (broken line).

rameters AUC/D and Pl Cl were remarkably similar. The large variations observed in half-life and Vd reflect the high variability of these parameters as reflected in the CV of these terms.

The individual values of kinetic parameters in five patients with renal insufficiency and in six with liver metastases are shown in tables 2 and 3. As with DX treatments, doses were decreased, except in patient 17 (liver metastasis; normal bilirubin level; high values for alkaline phosphatase, SGOT, and SGPT), who received the full dose of 90 mg/m^2. In this patient, 4′-epiDX and 4′-epiDXol plasma levels were consistently higher than in other patients treated with the same dose (fig 1).

The plasma-level profile of the drug and its metabolite in the patients treated with a reduced dose was qualitatively similar to that observed in patients with normal renal and hepatic functions. In patients with renal insufficiency (table 2), the mean AUC corrected for the dose was higher (48.3 vs 34.8) and the mean Pl Cl was lower (0.68 vs 0.88 L/min) than in patients with normal liver and kidney functions. However, examination of individual data reveals that the values for patients with renal impairment are within the same range as those observed in the group with normal renal function in all but one patient (No. 14, whose mean AUC/D and Pl Cl values were about three times higher and one-third lower, respectively, than the mean normal values). No important differences were found in the plasma half-life and Vd.

In the group of patients with liver metastases (table 3), Pl Cl was significantly lower than in patients with normal hepatic function (a mean value of 0.51 vs 0.88 L/min; $P = 0.0065$, Mann-Whitney and Kruskal-Wallis tests) (13), but AUC/D was larger (66.19 vs 34.84; $P = 0.02$, Mann-Whitney and Kruskal-Wallis tests). This high mean AUC/D value is in part due to the value for patient 17, who was treated with a normal dose. As for the other pharmacokinetic parameters (terminal plasma half-life, Vd γ, and AUC ratio), the differences between the two groups were negligible and without statistical significance. In particular, the half-life was not increased.

It is of particular interest that three patients without apparent hepatic lesions and with normal bilirubin levels had high AUC/D values and low Pl Cl values. These patients actually had rather high levels of alkaline phosphatase (patients 1 and 10) or transaminases (patient 5).

No clear-cut correlation between AUC/D or Pl Cl value and any single liver function test was found; however, all possible subset regression analysis (BMDP 9R program) showed a significant ($P = 0.0013$, ANOVA) multiple correlation between AUC/D and levels of SGOT and bilirubin (13).

Hematologic toxicity was mild to moderate and was usually reversible within 3 weeks after drug administration. No correlation was found between the AUC and the wbc count nadir. Only four of 22 patients had a wbc count nadir $< 2 \times 10^3$/mm^3, with the lowest value (0.5×10^3/mm^3) observed in the patient (No. 17) with liver metastases treated with the full dose of 90 mg/m^2.

Of 12 patients evaluable for response, four had partial remissions (patients 4, 5, 10, and 11) while two had minimal remissions (patients 9 and 17).

DISCUSSION

The results of this study confirm that the disposition of 4′-epiDX after administration in man is qualitatively similar to that of DX. The similarities include the typical triexponential plasma level profile of the unaltered drug, having a very long terminal plasma half-life, as well as the time course of the plasma levels of the reduced 13-hydroxy metabolite (14–17). This disposition is interpreted in terms of the classical multicompartment model, which has a high-capacity deep compartment, primarily responsible for the slow elimination of the drug.

Pharmacokinetic parameters demonstrated a rather broad interpatient spread, particularly evident in the terminal plasma half-life and Vd γ (CV of about 50%). Among patients with impaired and normal renal function, no significant differences were found in the disposition of either the drug or its metabolite. As with DX, renal excretion of 4′-epiDX and 4′-epiDXol comprises only a small part of the total elimination of both compounds, so dose modification is not mandatory if renal failure occurs.

On the contrary, both a statistically significant increase of the AUC (corrected for the dose) and a decrease of Pl Cl were found in the group of patients with liver metastases, in accordance with the predominant hepatic elimination of the drug. The terminal plasma half-life remained within the range of values observed in patients with normal liver function; this agrees with the findings of Perrier and Gibaldi (18), who demonstrated that clearance and not the long half-life term is a direct measure of the intrinsic metabolic activity of the liver, if the liver is an integral part of the central compartment.

No evidence was found of either altered formation or altered elimination of 4′-epiDXol, which contradicts the results of a pharmacokinetic study using DX in hepatoma patients (19). This finding suggests that a reduction to about 50% of the usual dose of 4′-epiDX is necessary to reduce to a minimum the possibility of aggravated toxicity (in cancer patients with liver metastases).

The reduced dose of 4′-epiDX prevented any strong hematopoietic suppression in five patients with liver metastases, even in the two having very high bilirubin levels (5.2 and 10.8 mg/100 ml), but severe leukopenia (0.5×10^3 wbcs/mm^3) occurred in the patient (No. 17)

TABLE 1.—Main pharmacokinetic parameters of 4′-epiDX in patients with normal hepatic and renal functions*

Patient No.	Dose mg/m²	Dose mg	AUC/D†	Pl Cl, L/min	Terminal plasma half-life, hrs	Vd γ, L/kg	AUC for 4′-epiDXol
1	90	170	53.50	0.588	64.6	43.8	0.508
2	90	150	35.16	0.948	29.0	26.4	0.207
3	90	160	22.71	1.304	19.0	28.8	0.260
4	70	121	47.57	0.606	34.4	43.3	0.172
5	90	130	41.05	0.586	29.4	30.8	0.282
6	90	150	25.41	1.093	14.3	20.5	0.208
7	90	145	28.20	0.952	75.1	99.3	0.220
	(60)	(100)‡	(23.91)	(1.161)	(24.2)	(39.0)	(0.156)
8	90	150	26.96	1.030	30.8	46.1	0.214
	(90)	(150)‡	(23.54)	(1.179)	(42.5)	(73.0)	(0.091)
9	90	160‡	27.81	1.065	57.0	77.4	0.147
10	90	140‡	44.20	0.586	30.7	25.7	0.341
11	60	150‡	30.70	0.950	55.4	65.1	0.224
		Mean	34.84	0.882	40.0	46.1	0.253
		SE	3.08	0.075	5.95	7.49	0.030
		CV	29.33	28.40	49.4	53.9	39.3

*Values in parentheses were excluded from calculation of the mean.

†$AUC/D = \frac{(ng \times hr)/ml}{mg/m^2}$

‡4th treatment course.

TABLE 2.—Main pharmacokinetic parameters of 4′-epiDX in patients with impaired renal function

Patient No.	Dose mg/m²	Dose mg	AUC/D*	Pl Cl, L/min	Terminal plasma half-life, hrs	Vd γ, L/kg	AUC for 4′-epiDXol
12	50	75	41.40	0.603	22.3	21.2	0.163
13	50	85	34.42	0.823	31.2	28.2	0.311
14	50	80	95.98	0.278	32.0	12.3	0.094
15	35.7	50	49.44	0.472	84.9	82.6	0.150
16	50	75	20.52	1.218	24.9	48.6	0.233
		Mean	48.35	0.678	39.1	38.6	0.190
		SE	12.82	0.161	11.6	12.5	0.037

*$AUC/D = \frac{(ng \times hr)/ml}{mg/m^2}$

TABLE 3.—Main pharmacokinetic parameters of 4′-epiDX in patients with liver metastasis

Patient No.	Dose mg/m²	Dose mg	AUC/D*	Pl Cl, L/min	Terminal plasma half-life, hrs	Vd γ, L/kg	AUC for 4′-epiDXol
17	90	135	103.93	0.240	21.1	8.4	0.362
18	70	95	34.18	0.661	31.2	42.5	0.180
19	50	80	47.04	0.567	51.8	41.6	0.200
20	50	85	125.26	0.226	29.6	9.2	0.468
21	50	74	58.34	0.423	29.5	19.5	0.318
22	50	80	28.44	0.937	28.5	41.3	0.187
		Mean	66.19	0.509	31.9	27.1	0.285
		SE	16.11	0.111	4.2	6.8	0.047

*$AUC/D = \frac{(ng \times hr)/ml}{mg/m^2}$

who was treated with 90 mg/m^2. However, this patient, who had normal bilirubin levels (0.8 mg/100 ml), was exposed to very high 4′-epiDX plasma levels (three to four times higher than those in normal patients treated with the same dose, in terms of AUC). The results of the present study do not permit any conclusion regarding the relationship between impaired liver function, 4′-epiDX pharmacokinetics, and drug effects. However, in view of both the previously discussed findings and the similar disposition of 4′-epiDX and DX, dose modification should be recommended in patients with signs of liver disorders as well as in those with liver metastases.

Further studies are necessary to elucidate the fate of 4′-epiDX after administration in man, with particular reference to the routes of excretion, the metabolic pathway, and the relative contributions of metabolism and biliary and renal excretion to the drug clearance. Such knowledge will assist in selecting the particular tests of liver function that can best account for major impairment in the clearance process of the drug and can predict the changes in drug pharmacokinetics.

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Modulation of Cytarabine Metabolism in the Human Promyelocytic Leukemia Cell Line HL-60 by Polyhydroxy-Substituted Benzohydroxamic Acids[1,2]

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Two potent new ribonucleotide reductase inhibitors, 3,4,5-trihydroxybenzohydroxamic acid (VF 122) and 3,4-dihydroxybenzohydroxamic acid (VF 147), were investigated for their ability to modulate the cellular pharmacology of cytarabine (ara-C) in HL-60 cells. VF 122 and VF 147 increased the total cellular uptake of ara-C by a mean (± SE) of 8% ± 3% and 29% ± 3%, respectively, when measured 2 hours after the start of exposure to 0.1 μM ara-C. This effect was evident after only 10 minutes of exposure to the ribonucleotide reductase inhibitor and did not vary significantly over the concentration range of 10–100 μM for either agent. VF 122 enhanced the incorporation of ara-CTP into DNA by 3.6-fold; VF 147 produced a 5.6-fold increase. In comparison, the maxiumum enhancement achievable with hydroxyurea was 2.1-fold, and with thymidine was 1.8-fold. These results provide a biochemical rationale for further investigation of these agents in combination with ara-C. [Cancer Treat Rep 66:1825–1829, 1982]

Cytarabine (ara-C) is one of the most important drugs currently available for the treatment of acute leukemia in man (1). The phosphorylation of ara-C by dCyd kinase is the rate-limiting step in the conversion of ara-C to its metabolite ara-CTP (2). Ara-CTP is a potent inhibitor of DNA polymerase in vitro (3,4) but in whole cells is also incorporated into DNA (5–9). The cytotoxicity of ara-C does not correlate well with its ability to inhibit DNA synthesis (10–13). However, it does correlate well with the amount of ara-C incorporated into DNA (8,9), and with the production of chromatid breaks (10,11). The extent of ara-CTP incorporation into DNA should be a function of the amount of ara-CTP formed, and recent work on the cellular pharmacology of ara-C in freshly harvested leukemic blasts demonstrated a correlation between the clinical response to ara-C and the extent of formation and retention of ara-CTP (14).

The effectiveness of ara-C is also influenced by the concentration of the normal substrate dCTP, with which ara-CTP must compete for binding to DNA polymerase (3,4,15,16). Phosphorylation of ara-C is normally constrained by feedback inhibition by dCTP (2,17). Theoretically, any drug that can reduce the intracellular concentration of dCTP should simultaneously enhance the phosphorylation of ara-C, increase the effectiveness of ara-CTP in inhibiting DNA polymerase, and increase the probability that ara-C rather than dCyd will be incorporated into DNA. One technique for accomplishing this is to inhibit the enzyme ribonucleotide reductase, which carries out an obligatory step in the de novo synthesis of dCTP (18).

Both thymidine (dThd) and hydroxyurea (HU) are inhibitors of ribonucleotide reductase (18–22). Both have been shown to increase the incorporation of ara-CTP into DNA (2,23,24) and to interact synergistically with ara-C with respect to cytotoxicity (23,25–30). Recently, Van't Reit et al (31) have synthesized a series of polyhydroxy-substituted benzohydroxamic acids which are 20–50 times more potent than HU as inhibitors of ribonucleotide reductase. The aim of this research was

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to determine whether even greater enhancement of ara-CTP incorporation into DNA could be achieved with these new agents.

MATERIALS AND METHODS

Cell Lines

HL-60 cells obtained from Dr. S. J. Collins were seeded at 5×10^5 cells/ml in RPMI-1640 medium with 10% heat-inactivated fetal calf serum, 1% antibiotic solution (penicillin at 10,000 units/ml, fungizone at 25 μg/ml, and streptomycin at 10,000 μg/ml; Flow Laboratories, McLean, VA), and 1% glutamine at 37°C in 5% CO_2. The cultures were allowed to grow for 1 day to 0.9–1.0 $\times 10^6$ cells/ml before being used in experiments.

Drugs

The two compounds, 3,4-dihydroxybenzohydroxamic acid (VF 147) and 3,4,5-trihydroxybenzohydroxamic acid (VF 122), were obtained from Dr. Bart Van't Reit, Medical College of Virginia, Richmond.

Cells (10^6/ml) were exposed to 10–100 μM VF 122 and VF 147 for varying times prior to the start of exposure to 0.1 μM ^{3}H-ara-C ([5-^{3}H]1-β-D-arabinofuranosylcytosine, 10.2 Ci/mmol; New England Nuclear, Boston). After the start of ^{3}H-ara-C exposure, aliquots of cells were removed at various times, washed twice with ice-cold phosphate-buffered saline, and precipitated with perchloric acid, and then both the acid-soluble and acid-insoluble fractions were counted by liquid scintillation. Incorporation of ^{3}H-ara-C into ara-CTP was determined by extracting 1-ml aliquots of cells with 0.4 M perchloric acid, neutralizing the extract with alamine and freon, and then separating ara-CTP from other forms of ara-C on polyethylene amine thin-layer chromatographic sheets (Brinkman, Westbury, NY). The sheets were run in 50% methanol, dried, and then run in 1 M LiCl. Labeled ara-CTP spots were located by UV light after adding cold ara-CTP (Calbiochem, San Diego). The ara-CTP spots were then cut out and counted in a liquid scintillation counter.

The extent of ^{3}H-ara-C incorporation into DNA was measured using the technique described by Kufe et al (8). Ten million cells (10^6/ml) were exposed to 100 μM VF 122 or VF 147 for 30 minutes and then simultaneously exposed to the ribonucleotide reductase inhibitors plus 0.1 μM ^{3}H-ara-C and 10 μCi/ml of $^{32}PO_4$ (carrier free, New England Nuclear) for an additional 2 hours. The cells were then digested with pronase B (Calbiochem) and extracted with phenol. The DNA was separated from RNA by banding the extract on a cesium sulfate gradient. Trichloroacetic acid precipitates of 1-ml aliquots were counted by liquid scintillation.

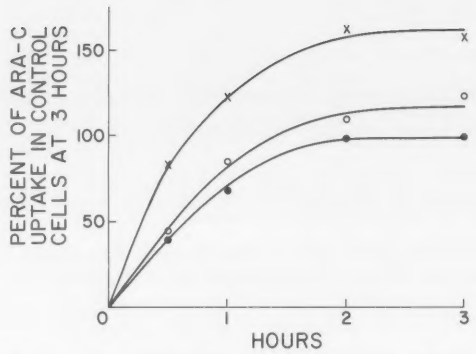

FIGURE 1.—Effect of 100 μM VF 122 (○) and VF 147 (×) on the total cellular uptake on ^{3}H-ara-C in HL-60 cells. Data are expressed as % of the uptake in control cells (●) after a 3-hr incubation with media alone. Cells were pretreated with VF 122 and VF 147 for 30 mins prior to the start of exposure to 0.1 μM ^{3}H-ara-C.

RESULTS

A concentration of 110 μM VF 122 reduced the growth rate of HL-60 cells by 50%; the corresponding concentration of VF 147 was 44 μM. The effect of each of these agents on the cellular uptake of ara-C and on the rate of incorporation of ara-CTP into DNA was studied at approximately equitoxic concentrations and compared to that of HU and dThd.

Figure 1 shows the effect of optimal concentrations of VF 122 and VF 147 on the total cellular uptake of ^{3}H-ara-C as a function of time. The ara-C concentration was 0.1 μM, a concentration commonly achieved by constant infusion during the treatment of patients with acute leukemia (33). For both of these drugs, as for HU and dThd (data not shown), the rapid phase of ^{3}H-ara-C uptake was complete by 2 hours. When HL-60 cells were preincubated with ribonucleotide reductase inhibitor at the optimal concentration for 30 minutes prior to the start of ^{3}H-ara-C exposure, VF 122 increased uptake by a mean (± SE) of 8% ± 3% at 2 hours. VF 147 produced a mean (± SE) increase of 29% ± 3% under the same conditions. In contrast, even without the 30-minute preincubation, HU and dThd produced a 300%–400% increase in ara-C uptake (34) at their optimal concentrations. Both VF 122 and VF 147 were tested at concentrations of 10–100 μM, but there was no significant variation in their effect on the cellular metabolism of ^{3}H-ara-C over this range.

Enhancement of ara-C uptake by HL-60 cells may be due to either an increase in conversion of ara-C to ara-CTP and incorporation into DNA or accumulation of cells in S phase. Although HU and dThd cause synchronization of cells in S phase, and a similar effect is expected from VF 122 and VF 147 based on their

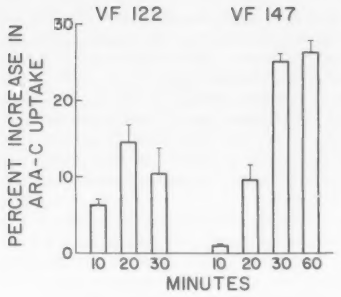

FIGURE 2.—Effect of the duration of pretreatment of HL-60 cells with 100 μM of VF 122 (left) and VF 147 (right) on the rate of total cellular ara-C uptake over the 1st 30 mins following the start of exposure to 0.1 μM ^{3}H-ara-C. Vertical bars = SD.

mechanisms of action, the data presented in figure 2 effectively exclude synchronization as an explanation for the change in the cellular metabolism of ara-C. The doubling time of HL-60 is 24 hours, whereas the effect of both VF 122 and VF 147 was apparent within 10 minutes of the start of drug exposure.

Table 1 shows the influence of each agent on the rate of ara-CTP incorporation into DNA at a concentration of the ribonucleotide reductase inhibitor which enhanced ara-C uptake. All four agents slowed the rate of DNA synthesis as evidenced by a reduction in the rate of incorporation of $^{32}PO_4$ into DNA. Despite this, all four agents increased the rate of ara-CTP incorporation per unit of newly synthesized DNA. The values given for the degree of enhanced incorporation for VF 122, VF 147, and dThd are for the optimal concentration of each agent; the degree of enhancement of ara-C uptake produced by HU continued to increase at HU concentrations > 1000 μM. Thus, despite the fact that VF 122 and VF 147 were not as effective as HU and dThd at increasing the cellular uptake of ara-C, they were more effective in promoting the incorporation of ara-CTP into DNA.

DISCUSSION

The polyhydroxy-substituted benzohydroxamic acids are primarily of interest because they are potent inhibitors of ribonucleotide reductase. When tested against ribonucleotide reductase from the Novikoff hepatoma, VF 122 was 50-fold and VF 147 was 20-fold more potent than HU (35). In the murine L1210 leukemia system, both drugs were more potent than HU and were at least as effective as HU in improving the lifespan of treated animals (35). In previous investigations in this laboratory, we have established that both dThd and HU can modulate the cellular pharmacology of ara-C to increase its incorporation into DNA, and that the mechanism of this effect in HL-60 cells is a marked reduction in the size of the dCTP pool due to inhibition of ribonucleotide reductase (34). The results of this study indicate that VF 122 and VF 147 are also capable of enhancing the cellular uptake of ara-C and the incorporation of ara-CTP into DNA. Although we have not specifically investigated the effects of VF 122 and VF 147 on the ribonucleotide reductase from HL-60 cells, there is nothing in the data that is inconsistent with the hypothesis that VF 122 and VF 147 are modulating the cellular metabolism of ara-C via inhibition of this enzyme.

A comparison of all four ribonucleotide reductase inhibitors points out an interesting dissociation between their effect on total cellular uptake and incorporation into DNA. HU and dThd, at their optimal concentrations, produced a doubling of the incorporation per unit of newly synthesized DNA (34). In contrast, VF 122 and VF 147 produced a greater effect on the incorporation of ara-CTP into newly synthesized DNA, but only an 8% and 29% increase, respectively, in total cellular uptake of ara-C. In the case of dThd and HU we have previously reported that the increase in total cellular uptake of ara-C is very closely matched by an increase in the ara-CTP pool size (34). We have not investigated the effect of VF 122 and VF 147 on the relative ara-CTP and dCTP pools of HL-60 cells; however, it has been shown that these compounds will lower the pools of dCTP in HeLa cells (36). It is possible that these drugs affect the distribution of total cellular ara-C among its various phosphorylated metabolites. To our knowledge, no information is available on the effect of these compounds on deoxycytidylate deaminase, whose activity can potentially influence the incorporation of

TABLE 1.—Effect of ribonucleotide reductase inhibitors on incorporation of ^{3}H-ara-C and $^{32}PO_4$ into DNA

Ribonucleotide reductase inhibitor	% of control ^{3}H-ara-C incorporated into DNA	% of control $^{32}PO_4$ incorporated into DNA	Ratio of ^{3}H-ara-C/ $^{32}PO_4$ incorporated into DNA
VF 122 (100 μM)	163	45	3.6
VF 147 (100 μM)	175	31	5.6
HU (1000 μM)	25	14	2.1
dThd (1000 μM)	129	62	1.8

ara-CTP into DNA by converting ara-CMP to ara-UMP (37–40).

The major significance of the ability of VF 122 and VF 147 to enhance the incorporation of ara-CTP into DNA lies in the recent demonstration by Kufe and his colleagues (8) and Major et al (9) that the cytotoxicity of ara-C correlates very well with DNA incorporation in HL-60 cells and freshly harvested acute leukemic myeloblasts. Using the same technique and cell line as used in this study, they have shown that ara-C is incorporated into internucleotide linkage as ara-C rather than ara-U or other metabolites (9). On the other hand, it has been known for some time that the cytotoxicity of ara-C does not correlate well with its ability to inhibit DNA synthesis (10–13). We and others (23,25–30) have previously reported that dThd and HU are synergistic with ara-C in various systems at concentrations that increase ara-C incorporation into DNA. It remains to be demonstrated that VF 122 and VF 147 behave the same way, but if so, they may find utility in combination with ara-C for the treatment of acute leukemia.

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Antiemetic Activity of Butorphanol Against Cisplatin-Induced Emesis in Ferrets and Dogs[1]

John E. Schurig,* Alexander P. Florczyk, William C. Rose, and William T. Bradner[2,3]

The analgesic agent butorphanol was evaluated for its ability to block cisplatin-induced emesis in ferrets and dogs. In ferrets, butorphanol (0.15, 0.3, or 0.45 mg/kg; expressed in terms of the tartrate salt) administered sc 30 minutes prior to cisplatin (8 mg/kg iv) reduced the number of emetic episodes but did not eliminate them. When these doses of butorphanol were administered 30 minutes before and 30 and 90 minutes after cisplatin, they caused a dose-related reduction in the number of emetic episodes; there was complete protection at a dose of 0.45 mg/kg/injection. In dogs, butorphanol (0.185 or 0.37 mg/kg/injection, expressed in terms of the tartrate salt) was administered sc on the same multiple-dose schedule used in the ferrets; this caused a nearly complete elimination of the emetic response to cisplatin (3 mg/kg iv). Butorphanol caused some sedation in both species at effective antiemetic doses but had no effect on the antitumor activity of cisplatin against murine L1210 leukemia. Naloxone blocked the antiemetic effect of butorphanol in ferrets, indicating the involvement of opiate receptors. The results of these studies suggest that butorphanol may be useful clinically in mitigating the emetic effects of cisplatin. [Cancer Treat Rep 66:1831–1835, 1982]

The nausea and vomiting induced by cancer chemotherapeutic agents is a common and distressing problem for cancer patients. Prolonged nausea and vomiting can have serious consequences on nutrition, metabolism, and the quality of life of such patients and has caused some of them to reject potentially lifesaving drug treatment (1–3). The currently available antiemetics such as the phenothiazines, antihistamines, and butyrophenones offer some relief but are generally considered to be inadequate against the more emetogenic chemotherapeutic agents (4–7). Consequently, there is a great deal of interest in the development of new antiemetic drugs that are more effective against cancer chemotherapeutic agents.

Previous studies by Florczyk et al (8) and Gylys et al (9) have shown that ferrets and dogs vomit repeatedly beginning approximately 60–90 minutes after cisplatin administration. We now report the results of our evaluations of the antiemetic effects of butorphanol, an agonist-antagonist analgesic agent (10), against cisplatin-induced emesis in these animals. We also evaluated the effect of butorphanol on the antitumor activity of cisplatin against murine L1210 leukemia.

MATERIALS AND METHODS

Animals

The antiemetic studies were performed in conscious male Fitch ferrets (1–1.5 kg) and beagle dogs of either sex (9–12 kg). To facilitate iv dosing, each ferret had an indwelling jugular catheter surgically implanted 2–4 days prior to use, as previously described (11). Female CDF_1 mice (16–19 g) were used in the antitumor studies. All animals were given free access to feed and water.

Antiemetic Studies

In ferrets, butorphanol (0.15, 0.3, or 0.45 mg/kg/injection) was injected sc in the scapular region 30 minutes prior to the administration of cisplatin (8 mg/kg iv). Additional doses of butorphanol were administered sc 30 and 90 minutes after cisplatin in the multiple-dose

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studies. One group of ferrets was treated with butorphanol (0.45 mg/kg sc) 30 minutes prior to cisplatin and then with naloxone (2 mg/kg sc) 45 minutes after cisplatin. In dogs, butorphanol (0.185 or 0.37 mg/kg/injection) was injected sc in the scapular region 30 minutes before and 30 and 90 minutes after the administration of cisplatin (3 mg/kg iv). The emetic responses of cisplatin, consisting of retching and expulsion as described (in cats) by McCarthy and Borison (12), were determined in both species by direct observation of the animals during a period lasting 4–6 hours after cisplatin administration. The onset time of the first emetic episode and the number of episodes were recorded for each animal. A subjective assessment (minimal, mild, moderate, or marked) was made of any behavioral effects of butorphanol.

Antitumor Studies

CDF_1 mice were implanted ip with 10^6 L1210 leukemia cells derived from ascites fluid produced in DBA/2 mice. On Day 1 after implant, the mice (six per group) received either a single ip injection of cisplatin, three sc injections of butorphanol 1 hour apart, or both drugs. When both drugs were administered, butorphanol was given sc 30 minutes before and 30 and 90 minutes after ip cisplatin. The effect of each drug treatment was judged on the basis of the relative increases in median survival time (MST) compared to MST in untreated control mice; this parameter was expressed as % T/C, which represents the MST of a drug-treated group divided by the MST of the control mice, × 100. The doses were determined according to the mean body weight of each group of mice. Cisplatin was administered at 12-, 8-, 6-, and 4-mg/kg dose levels, and butorphanol was administered at doses ranging from 0.6 to 60 mg/kg/injection.

Drugs

In the antiemetic studies, butorphanol was administered as the clinically used parenteral solution (Stadol) or as 0.9% NaCl dilutions of this solution. For the antitumor studies the butorphanol tartrate salt was dissolved in 0.9% NaCl. The doses of butorphanol in all studies were expressed in terms of the tartrate salt. The cisplatin solutions used in the antiemetic studies, 4 mg/ml for ferrets and 3 mg/ml for dogs, were prepared by adding heated (70°C) 0.9% NaCl to the cisplatin, stirring and sonicating until the cisplatin was in solution, and then maintaining the solution at 40°C for administration within 30 minutes. For the antitumor studies, cisplatin was dissolved in 0.9% NaCl without heating, since the concentrations used were ≤ 1 mg/ml. Naloxone was dissolved in 0.9% NaCl and the dose was expressed in terms of the HCl salt.

RESULTS

The results of the single-dose butorphanol studies in ferrets are summarized in table 1. In the control animals (no butorphanol treatment), cisplatin caused 7.4 ± 0.8 (mean ± SE) emetic episodes beginning about 70 minutes after administration. Butorphanol was administered only once, 30 minutes prior to cisplatin. The ferrets pretreated with butorphanol (0.15 and 0.3 mg/kg) had about one-half the number of emetic episodes as the control animals, with some apparent reduction in onset time of emesis; however, there was considerable variability in the number of episodes at these doses, with one of the four animals that received a dose of 0.3 mg/kg having no emesis. The ferrets pretreated with butorphanol at a dose of 0.45 mg/kg had a marked reduction in the number of emetic episodes (0.9 ± 0.5), with five of eight animals experiencing complete protection. The other three ferrets had 2.7 ± 0.3 emetic episodes, which is about one-third the number in the control animals. The behavioral effects of butorphanol consisted of minimal to mild sedation in some animals, which was not dose-related and was difficult to assess reliably due to the normally sedentary behavior of the ferret during daylight hours.

TABLE 1.—Antiemetic activity of single-dose butorphanol tartrate against cisplatin-induced emesis in ferrets

Butorphanol tartrate (mg/kg sc)*	No. with emesis/No. tested	No. of episodes†	Emesis onset in responding animals (mins)†
Control	12/12	7.4 ± 0.8	66.8 ± 3.9
0.15	4/4	3.3 ± 1.1‡	47 ± 5.4‡
0.30	3/4	3.5 ± 2.2 (4.7 ± 2.7)	41.7 ± 0.9‡
0.45	3/8	0.9 ± 0.5‡ (2.7 ± 0.3)‡	81 ± 4.6

*Single dose administered 30 mins prior to cisplatin (8 mg/kg iv).
†Values = mean ± SE. Values in parentheses = No. of episodes in responding animals.
‡$P < 0.05$, significantly different from control; Student's t test.

A summary of the results from the multiple-dose butorphanol studies is shown in table 2. The controls from the single- and multiple-dose studies were pooled since many of the tests were done concurrently. Therefore, the control data in table 2 are the same as in table 1. Butorphanol administered on the multiple-dose schedule caused a dose-related reduction in the number of emetic episodes, with complete protection in one animal at a dose of 0.3 mg/kg/injection and in all animals at a dose of 0.45 mg/kg/injection. There was essentially no effect on onset time of emesis in the animals responding to cisplatin. The behavioral effects of butorphanol observed in these studies consisted of sedation, which was minimal at the low dose, mild at the intermediate dose, and mild to moderate at the high dose.

The results of the evaluation of the antiemetic activity of butorphanol in dogs are shown in table 3. Cisplatin (3 mg/kg) caused emesis in the control dogs, with a mean of 14 ± 3 emetic episodes beginning about 100 minutes after dosing. Butorphanol administered at a dose of 0.185 or 0.37 mg/kg/injection 30 minutes before and 30 and 90 minutes after cisplatin markedly reduced the number of emetic episodes. Only one of the three dogs in each butorphanol dose group vomited, and each had only two episodes. The onset times were comparable or slightly longer than in the control animals. The behavioral effects of butorphanol consisted of minimal to mild sedation at the low dose and mild to marked sedation at the high dose.

Table 4 summarizes the results of a study to determine if butorphanol had any effect on the antitumor activity of cisplatin against murine L1210 leukemia. Cisplatin alone produced a maximum T/C of 171% at a dose of 8 mg/kg ip. Butorphanol administered sc at doses of 0.6–60 mg/kg/injection, on the same multiple-dose schedule used in the emesis studies, had no effect on the antileukemic activity of cisplatin as determined at optimal dose levels, nor did butorphanol exhibit any antileukemic activity of its own.

The results of studies done to evaluate the effect of naloxone on the antiemetic activity of butorphanol against cisplatin-induced emesis in ferrets are shown in table 5. In the control animals, cisplatin caused 6.7 ± 0.5 emetic episodes with an onset time of about 70 minutes. Butorphanol (0.45 mg/kg) administered as a single dose 30 minutes prior to cisplatin completely blocked the emetic effect of cisplatin in all three animals. In the third group of ferrets, butorphanol (0.45 mg/kg) was given 30 minutes prior to cisplatin, and naloxone (2 mg/kg) was given 45 minutes after cisplatin. These ferrets responded to the emetic effect of cisplatin, with an average of 14.5 emetic episodes. The last group of ferrets

TABLE 2.—Antiemetic activity of multiple-dose butorphanol tartrate against cisplatin-induced emesis in ferrets

Butorphanol tartrate (mg/kg/injection sc)*	No. with emesis/ No. tested	No. of episodes†	Emesis onset in responding animals (mins)†	Sedation
Control	12/12	7.4 ± 0.8	66.8 ± 3.9	—
0.15	3/3	3.7 ± 1.7	86.6 ± 27.2	Minimal
0.30	3/4	2.0 ± 0.9‡ (2.7 ± 0.9)	80.7 ± 16.2	Mild
0.45	0/4	0‡	—	Mild to moderate

*Administered 30 mins before and 30 and 90 mins after cisplatin (8 mg/kg iv).
†Values = mean ± SE. Values in parentheses = No. of episodes in responding animals.
‡$P < 0.05$, significantly different from control; Student's t test.

TABLE 3.—Antiemetic activity of butorphanol tartrate against cisplatin-induced emesis in dogs

Butorphanol tartrate (mg/kg/injection sc)*	No. with emesis/ No. tested	No. of episodes†	Emesis onset in responding animals (mins)†	Sedation
Control	3/3	14 ± 3.1	102 ± 10.4	
0.185	1/3	0.7 ± 0.7‡ (2.0)	112	Minimal to mild
0.37	1/3	0.7 ± 0.7‡ (2.0)	150	Mild to marked

*Administered 30 mins before and 30 and 90 mins after cisplatin (3 mg/kg iv).
†Values = mean ± SE. Values in parentheses = No. of episodes in responding animals.
‡$P < 0.05$, significantly different from control; Student's t test.

TABLE 4.—Effect of butorphanol tartrate on antileukemic activity of cisplatin against murine L1210 leukemia*

Butorphanol tartrate (mg/kg sc)	Cisplatin	
	Optimal dose† (mg/kg ip)	Maximum % T/C
0	8	171
0.6	6	171
1.5	8	179
6	8	179
15	6.8	171
60	6	164

*CDF_1 mice were implanted ip with 10^6 L1210 cells. Treatments were given 1 day after implant. Cisplatin was administered ip at doses of 12, 8, 6, and 4 mg/kg. Butorphanol was administered 30 mins before and 30 and 90 mins after cisplatin. There were 6 mice in each group. Butorphanol alone had no antileukemic effect.

†That dose achieving the greatest % T/C at acceptable levels of toxicity (not > 1 of 6 mice dying within 4 days of drug treatment).

received the treatment with butorphanol and naloxone without cisplatin and had no emetic episodes.

DISCUSSION

In these studies, butorphanol had antiemetic activity against cisplatin-induced emesis in both ferrets and dogs. This effect of butorphanol resulted in marked reductions in the number of emetic episodes and in some cases, complete protection. To our knowledge the antiemetic activity of butorphanol has not been described previously and therefore represents a novel effect for an agonist-antagonist type of analgesic agent. It has been shown that the narcotic analgesic agents such as morphine and fentanyl have antiemetic activity in cats and dogs (13,14). However, the narcotic analgesics are not often used clinically as antiemetics in cancer chemotherapy (7). Therefore, butorphanol could represent a new antiemetic agent for addition to the armamentarium used against emesis induced by chemotherapeutic agents. Due to its antagonist properties, however, the use of butorphanol would be restricted to patients having had no prior treatment with analgesics.

The use of ferrets to evaluate drugs for emetic and antiemetic activity is a new approach to this type of testing. Therefore, it was important to confirm the results from the ferret studies in the more widely recognized dog model. In a preliminary report on the use of the ferret as an animal model for emesis testing (8), we showed that the emetic effects of cisplatin in ferrets were similar to those described in dogs (9). This report also showed that metoclopramide, a drug that has antiemetic activity against cisplatin in dogs (9) and man (15,16), was an effective antiemetic in ferrets. The emetic activity of cisplatin and the antiemetic activity of metoclopramide in the animal models and man indicate good correlation between the results in the studies of the ferret and the dog and between the results of the studies of the two animal models and man, relative to their qualitative responses to these two drugs. Because butorphanol had antiemetic activity in both ferrets and dogs, the drug may be expected to have antiemetic activity in man.

The only behavioral effect of butorphanol that was observed in these studies was sedation. This side effect was anticipated since butorphanol can cause sedation in man (17). The degree of sedation appeared to be dose-related in both species in the multiple-dose studies.

An important consideration regarding the use of any toxicity-mitigating treatment in conjunction with a chemotherapeutic agent is the effect on antitumor activity. This is particularly critical with a chemically reactive drug like cisplatin. Butorphanol, at doses ranging

TABLE 5.—Effect of naloxone on the antiemetic activity of butorphanol tartrate against cisplatin-induced emesis in ferrets

Drug	Dose (mg/kg sc)	No. with emesis/ No. tested	No. of episodes*	Emesis onset in responding animals (mins)*
Control	—	7/7	6.7 ± 0.5	69.9 ± 4.0
Butorphanol	0.45†	0/3	0‡	—
Butorphanol + Naloxone	0.45† + 2.0§	2/2	14.5 ± 2.5‡	53.5 ± 2.5‡
Butorphanol + Nalozone¶	0.45 + 2.0	0/2	0	—

*Values = mean ± SE.

†Single dose administered 30 mins before cisplatin (8 mg/kg iv).

‡$P < 0.05$, significantly different from control; Student's t test.

§Single dose administered 45 mins after cisplatin (75 mins after butorphanol).

¶Butorphanol and naloxone administered as single doses, 75 mins apart, without cisplatin.

from 0.6 to 60 mg/kg/injection (30 minutes before and 30 and 90 minutes after cisplatin), had no effect on the antitumor activity of cisplatin against murine L1210 leukemia. Although this was a study of only one tumor type, the results suggest that butorphanol does not interfere with the antitumor activity of cisplatin.

The results with naloxone indicate that opiate receptors are involved with the antiemetic effect of butorphanol. The location of these opiate receptors is not known, but there are data to suggest that they are associated with the vomiting center rather than the chemoreceptor trigger zone (18). Assuming this location is correct, the antiemetic effect of butorphanol may be exerted centrally at the level of the vomiting center. However, additional studies are needed to establish the mechanism of the antiemetic action of butorphanol more adequately.

Naloxone not only blocked the antiemetic effects of butorphanol in ferrets but also appeared to augment the emetic responses to cisplatin (table 5). Treatment with butorphanol followed by naloxone (without cisplatin) was not emetic, indicating that naloxone was not unmasking a butorphanol-induced emesis. This suggests that endogenous opiates may be modulating the severity of the cisplatin emetic response. Additional studies are planned to address this possibility.

Based on results of these studies, butorphanol has become a candidate for clinical trial as an antiemetic agent against cisplatin-induced emesis. In preliminary studies in ferrets (data not shown), we found that iv doxorubicin caused emesis that was blocked by butorphanol. These studies are being expanded, but the initial results indicate that butorphanol has antiemetic activity against emetic cancer chemotherapeutic agents other than cisplatin.

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Response of the Dunning R3327H Prostatic Adenocarcinoma to Radiation and Various Chemotherapeutic Drugs[1]

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Dunning R3327H prostatic adenocarcinoma was bilaterally transplanted in the flanks of animals at the Papanicolaou Institute in Miami, and the animals were received at the Cross Cancer Institute (Edmonton, Alberta, Canada) each month. The animal flanks were palpated weekly, and when tumor volumes reached a size of approximately 300 mm^3 the animals were randomized into treatment groups for the assessment of various therapies. Tumor volumes were determined each week before and after various treatments, and tumor growth was compared to that in untreated controls. Ionizing radiation at relatively small single doses completely inhibits tumor growth for a period of up to 6 months. Some interesting characteristics of this radiation-induced growth arrest are that tumors do not die and shrink away as with some other tumor models but remain static in size and show histologic evidence of viable tumor cells. The hypoxic cell radiosensitizer misonidazole potentiates radiation response in this tumor model. Cisplatin, vincristine, etoposide, and estramustine phosphate administered in drug doses approaching their toxic limits have a partial effect on tumor growth. [Cancer Treat Rep 66:1837-1843, 1982]

Some physiologic and pathologic aspects of the Dunning R3327H rat prostatic cancer model have been described (1,2). This slow-growing, hormone-responsive tumor has similarities to human prostatic cancer and consequently is of interest in studies designed to test various treatment modalities. Research with this tumor model was initiated at the Cross Cancer Institute (Edmonton, Alberta, Canada) in 1979, and initial studies have involved an evaluation of the utility of this rat prostatic tumor in predicting the response of human prostatic cancer to various treatment modalities. We have measured the effectiveness of ionizing radiation with and without the hypoxic cell radiosensitizer misonidazole and of several chemotherapeutic drugs in current clinical use. All statements on tumor response in these initial experiments with R3327H are based on the effect of treatment on tumor volume growth kinetics.

MATERIALS AND METHODS

First-generation (F_1) hybrids of the matings of the Copenhagen male and Fischer female rats were injected sc in each flank with a 2-mm^3 core of R3327H tumor at the Papanicolaou Institute, Miami. These animals are supplied to various research centers courtesy of the National Prostatic Cancer Project in an effort to standardize treatment results. Implanted rats were flown to the Cross Cancer Institute and examined weekly until the tumor nodule was palpable (approximately 10–16 weeks). Each animal was individually caged and identified throughout the study. The tumor nodules were then measured weekly with calipers in three mutually orthogonal dimensions while the rats were under light halothane anesthesia. A double skin flap dimension of 1.3 mm was subtracted from each caliper reading, and tumor volumes were calculated using the formula $\frac{\pi}{6} \times d_1 \times d_2 \times d_3$, the formula for an ellipsoidal mass.

Animals were randomized into specific treatment protocols when at least one of their tumors reached a volume of 200–400 mm^3. The volumes of at least ten different tumors were averaged to indicate the tumor growth prior to and subsequent to a specific therapy. At least ten different untreated tumors served as normal growth controls at various times throughout the course of these studies, and maximum tumor doubling

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time for different control groups was between 9 and 12 days. Either the day of treatment (radiation therapy) or the first day of treatment (chemotherapy) was defined as Day 0, with prior and subsequent measurements of tumor volumes plotted as negative or positive times. The initial average volume of the treatment groups was chosen as the Day-0 point on the control curve. Wide variations were observed for times between implantation and the emergence of tumors to a volume of 200–400 mm^3.

Further analyses of the radiotherapy data were performed to establish the approximate log_{10} cell kill for a more meaningful interpretation of these results. The following formula was used (3):

$$\log_{10} \text{tumor cell kill/dose} = \frac{T - C}{(3.33)\,(Td)\,(\text{No. of doses})},$$

where T = time (days) for median tumor size in treated group to reach 1000 mm^3 volume; C = time (days) for median tumor size in control group to reach 1000 mm^3 volume; Td = tumor volume doubling time; and 3.33 = No. of tumor cell doublings/1 log.

Because the initial treatment volumes in the chemotherapeutic groups were slightly different, growth delays were calculated at a specific time after the treatment. Growth delays were determined at three different points on each tumor curve, representing the volumes at 4, 6, and 8 weeks after therapy and their delay after normalization of the curve with the control curve. Statistical comparisons of the treatment and control curves were performed by analyzing the growth delay utilizing the Student's t test.

Radiotherapy Procedures

R3327H tumors in the flanks of anesthetized rats were irradiated at a volume of 200–400 mm^3 with a Picker ^{137}Ce therapy unit. Single-dose fractions of 1000, 2000, and 2500 rad were applied. The radiation dose to each tumor was administered through two different ports to facilitate homogeneous dose distribution throughout the tumor. A 2-cm cone field was utilized with the tumor pulled away from the body wall, resulting in minimal scatter and low total-body irradiation. In concurrent experiments to study the effect of the hypoxic cell radiosensitizer misonidazole (0.5 mg/g), the drug was administered ip approximately 30 minutes prior to exposure of the tumors to 1000 and 1500 rad.

Chemotherapy Treatments

The drugs tested in these preliminary studies were of interest because they were already in clinical use for genitourinary malignancies or were being tested in phase II trials. Dosages were calculated on the basis of body surface area rather than body weight. Appropriate doses were determined using experience with human drug protocols as well as guidelines in product monographs. Where necessary, an approximate LD50 was determined by using the method of Freireich et al (4).

Misonidazole was administered at a dose level that was known to produce high serum and tissue concentrations of drug and was nontoxic in single doses to other strains of rats.

Chemotherapeutic drugs were administered ip using the following schedules:

(*a*) 2 mg/m^2 of vincristine 1 day/week for 3 weeks and 20 mg/m^2 of cisplatin on 3 alternate days within 1 week;

(*b*) 30 mg/kg/day of estramustine phosphate 5 days/week for 2 weeks;

(*c*) 75 mg/m^2 of teniposide on 3 alternate days for 1 week and 150 mg/m^2 of etoposide on 3 alternate days/week for 2 weeks; and

(*d*) an initial dose of 0.2 mg/g of misonidazole and 0.1 mg/g at 2 and 4 hours on 3 alternate days for 1 week.

All treatment groups included a minimum of ten tumors. Control groups were treated with ip injections of normal saline.

RESULTS

Control growth curves using a minimum of ten tumors consistently showed a doubling time of 9–12 days. Variation in tumor size is indicated by error bars in figure 1. All treatment curves are standardized at the time of initial treatment, using one control curve.

A dose of 1000 rad was sufficient to produce a significant growth delay of the R3327H tumor. This represented a log kill of approximately 1.5, with 2000 rad producing a kill of 3.4 (table 1). The effect of radiation on tumor growth appeared to be dose-dependent, with 2500 rad producing complete tumor growth arrest for

TABLE 1.—Radiotherapy*

Therapy	Time to tumor volume of 1000 mm^3 (days)	log_{10} cell kill (Td = 9 days)
Control	16	—
1000 rad	62	1.5
2000 rad	117	3.4
2500 rad	⩾ 154	⩾ 4.6
Misonidazole + 1000 rad	112	3.2
Misonidazole + 1500 rad	⩾ 168	⩾ 5.1

*Effects of radiotherapy are expressed in terms of log_{10} cell kill utilizing formula $\frac{T - C}{3.33\,(Td)\,(\text{No. of doses})}$. Assuming regrowth of tumor at 126 days in groups that had received either 2500 rad or misonidazole + 1500 rad, minimum log cell kill can be calculated.

4–6 months (fig 1). Assuming regrowth of the tumors at 126 days, the minimum log kill is 4.6. Tumors treated with 2500 rad showed no significant shrinkage after irradiation despite the successful arrest of total tumor growth. A histologic section of an R3327H tumor in which growth had been arrested by 2500 rad of ionizing radiation for 4 months is shown in figure 2. Zones of histologically viable adenocarcinoma are evident.

Addition of misonidazole before the radiation treatment enhanced this radiation response (fig 3). Misonidazole (0.5 mg/g) had a significant effect on the response of this tumor to radiation at a dose of 1000 rad (log kill 3.2), and the sensitized response to 1500 rad was approximately equivalent to that observed with 2500 rad alone, with a minimum log kill of 5.1 (table 1).

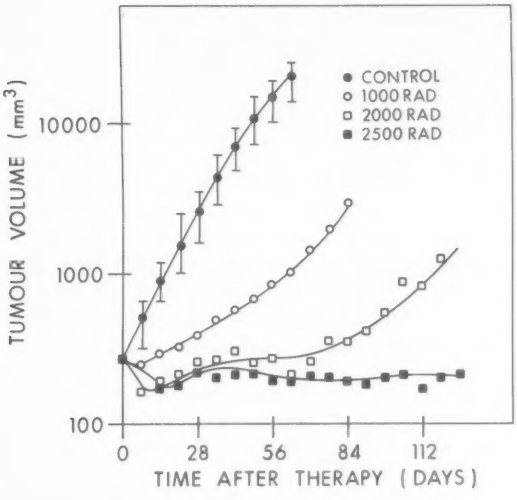

FIGURE 1.—R3327H tumor growth is inhibited with relatively small single doses of radiation. No tumor exhibited regrowth after 2500 rad; however, small palpable tumors remained. Error bars on control curve indicate both variability of tumor size and measuring error.

The chemotherapeutic drug regimens chosen resulted in moderate drug toxicity (mainly weight loss) (table 2). The drug teniposide was excessively toxic in the protocol tested and resulted in a low number of surviving animals that did not permit analysis of tumor growth with significance. The data shown in figure 4 indicate that significant growth delays ($P < 0.005$) were obtained with cisplatin, vincristine, and etoposide (table 3). The chemotherapeutic regimen with cisplatin had the greatest effect on tumor growth of the drugs tested, with an average growth delay of 4.5 weeks at 4–8 weeks after therapy. The amount of estramustine phosphate administered was relatively toxic, and the tumor growth delay that was observed (table 3) might correlate with the nutritional status of the animal, as evidenced by the severe weight loss (approximately 27%) induced by this drug (fig 5).

As a control to the irradiation studies using the hypoxic cell sensitizer misonidazole, a group of animals treated with drug alone were observed. The data of figure 6 indicate that multiple doses of the hypoxic radiosensitizer misonidazole that were sufficient to achieve adequate serum levels for several hours failed to affect the growth of the R3327H tumor in these rats.

DISCUSSION

External-beam radiotherapy is an accepted treatment modality for adenocarcinoma of the prostate in man (5,6). Fractionated-dose irradiation protocols with a total dose of up to 6000–7200 rad can result in a significant number of cures. Using the Ellis formula for nominal standard dose (NSD) (7), such clinical courses of radiotherapy produce an NSD of 1600–2000 ret. The NSD is the single-dose equivalent in rad required to produce the biologic response of the fractionated-dose irradiation protocol. Although direct extrapolation of human NSD values to experimental animal models cannot be made, the radiation doses utilized in these experiments are in a similar range. The data of figure 1 indicate that tumor volume increase is effectively controlled (for at least 6 months) by 2500 rad of radiation.

TABLE 2.—Effects of chemotherapy*

Drug	No. of— Animals	Deaths	Tumors analyzed	Mean initial tumor volume ($mm^3 \pm SD$)	Maximum mean % weight loss
Control	13	0	15	—	—
Vincristine	7	0	10	389 ± 126	7.8
Cisplatin	12	4	11	273 ± 89	18.5
Etoposide	9	0	25	303 ± 198	7.9
Teniposide	8	4	0	309 ± 182	22.3
Estramustine phosphate	7	1	14	348 ± 280	27.7
Misonidazole	8	0	14	183 ± 65	4.0

*Only teniposide proved to be excessively toxic in terms of animal deaths. Both cisplatin and estramustine phosphate resulted in substantial weight loss.

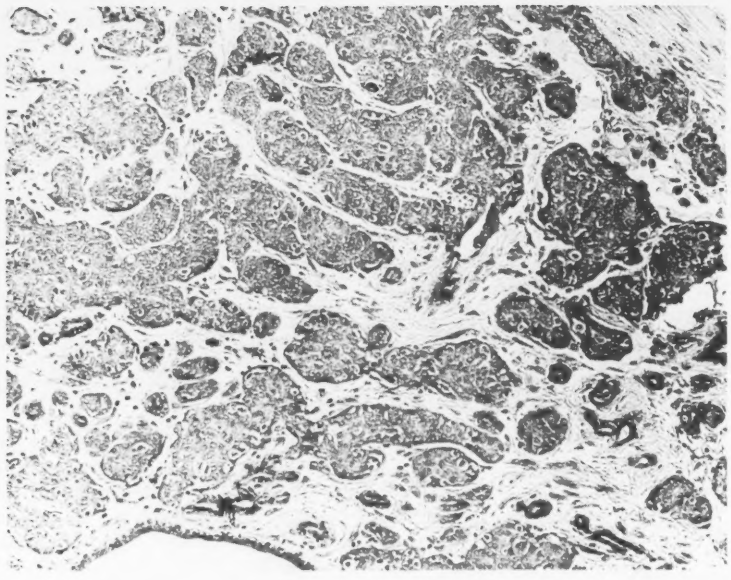

FIGURE 2.—H + E section (× 25) of an R3327H tumor 4 mos following 2500 rad. Histologic areas of apparently viable carcinoma are evident.

Single doses of 1000–2000 rad have a significant effect on tumor progression. Although increases in tumor volumes were effectively controlled with 2500-rad single doses, the tumor size was stable for several months after irradiation. Additional long-term experiments are now under way to determine the length of time this effect continues. A similar response of the R3327G tumor to ionizing radiation has been observed by Camuzzi et al (8). The persistence of palpable tumors may be related to the architecture of the tumor. R3327H is a moderately well-differentiated adenocarcinoma with a large amount of stromal tissue containing collagen that may retard tumor regression.

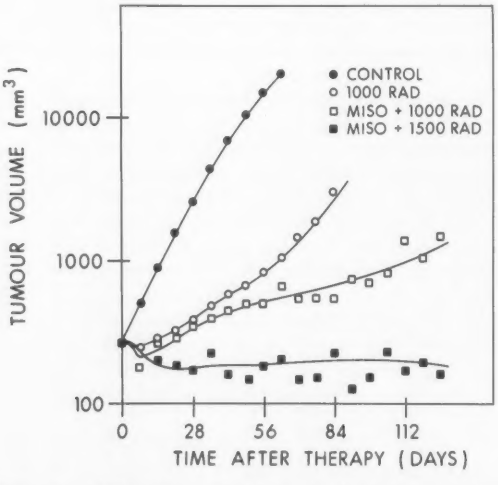

FIGURE 3.—Addition of the radiosensitizer misonidazole (0.5 mg/g) given 30 mins prior to treatment enhanced the effect of radiotherapy. Misonidazole (miso) + 1500 rad produced complete growth inhibition.

The finding that viable carcinoma is present in the nongrowing R3327H tumors for several months after treatment with 2500 rad of radiation appears to be analogous to the situation in man. The incidence of positive prostatic biopsies 1 year following attempted curative irradiation of human prostatic carcinoma is appreciable in most reports (9). Whether or not these histologically viable carcinoma cells observed in sections of irradiated R3327H tumor have proliferative and/or clonogenic potential is under current investigation. We have cultured, in vitro, an anaplastic cell line from the R3327AT tumor (courtesy of Dr. D. Coffey, Johns Hopkins University, Baltimore). Attempts will be made to culture in vitro viable cells from the hormonally responsive R3327H tumor so that cell proliferative capacity can be assayed directly.

The preliminary experiments performed with the hypoxic cell radiosensitizer misonidazole suggest that this rat tumor contains viable hypoxic cells that can play a

TABLE 3.—Tumor growth delays following chemotherapy*

Drug	Time after therapy (wks)	Growth delay (wks ± SD)	Average growth delay (wks)	*P* value at 8 wks
Vincristine	4	1.6 ± 0.9	2.3	< 0.005
	6	2.2 ± 1.3		
	8	3.0 ± 1.5		
Etoposide	4	1.3 ± 1.0	2.0	< 0.005
	6	2.1 ± 1.5		
	8	2.5 ± 1.9		
Cisplatin	4	3.3 ± 1.3	4.5	< 0.005
	6	4.6 ± 1.3		
	8	5.7 ± 1.9		
Estramustine phosphate	4	1.2 ± 1.4	2.7	< 0.005
	6	2.6 ± 1.9		
	8	4.2 ± 2.4		
Misonidazole	4	0.2 ± 0.9	—	—
	6	0.2 ± 1.3		
	8	0.4 ± 1.5		

*Cisplatin resulted in longest average growth delay over 4–8 wks after therapy. At 8 wks all growth delays were highly significant except with misonidazole, which showed virtually no cytotoxic effect.

role in tumor regrowth after treatment with relatively low doses of ionizing radiation. Additional studies need to be performed to precisely define sensitizer enhancement ratios as a function of drug dosage. Nevertheless, the administration of 0.5 mg/g of misonidazole 30–45 minutes prior to administration of radiation resulted in significant therapeutic enhancement. The data of figure 6 indicate that misonidazole administered alone does not have a direct cytotoxic effect on R3327H tumor cells.

Studies with cells in vitro (10) and with animal tumors in vivo (11) have indicated that hypoxia is required for this drug to have a radiosensitizing potential and/or a cytotoxic potential at the concentration of drug administered in these studies. ^{14}C-misonidazole has been used as a marker for hypoxic cells in solid tumors (12). Attempts to use ^{14}C-misonidazole to mark hypoxic cells in the R3327H tumor growing in rats have been unsuccessful to date. Examination of histologic sections of

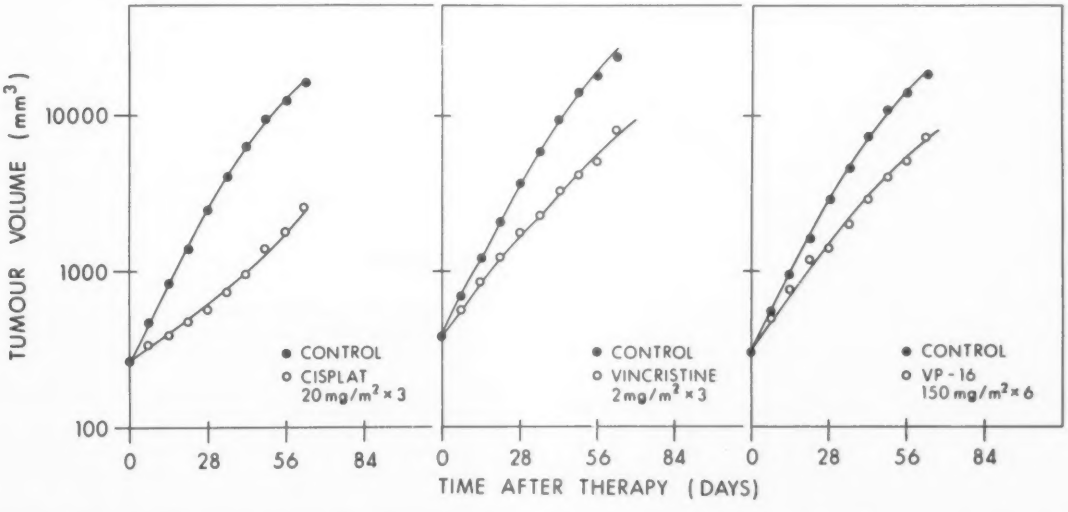

FIGURE 4.—Cisplatin (cisplat) produced a longer growth delay than either vincristine or etoposide (VP-16). All growth delays were significant at 4, 6, and 8 wks ($P < 0.005$).

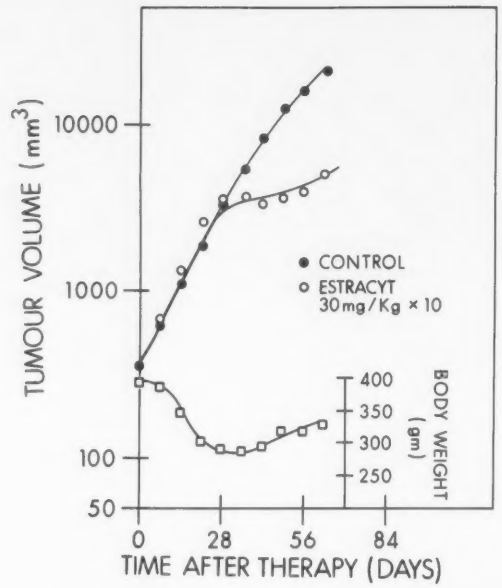

FIGURE 5.—Estramustine phosphate (Estracyt) was toxic. Significant growth delay was evident ($P < 0.005$); however, this was associated with a 27% weight loss. The role of nutrition in growth of R3327H tumor is undetermined.

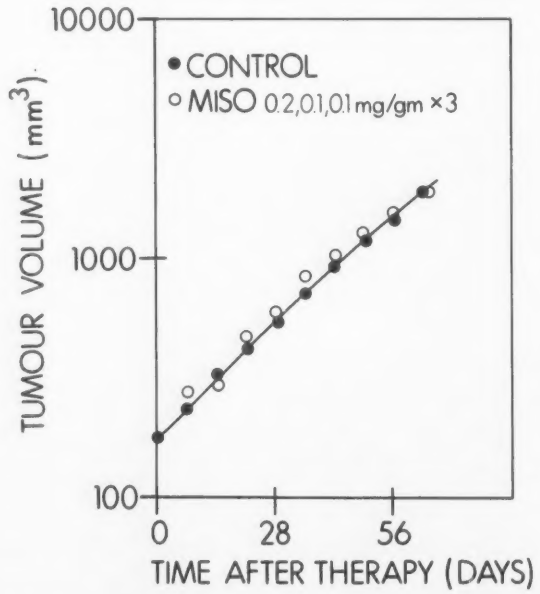

FIGURE 6.—Misonidazole (miso) given solely as a chemotherapeutic drug resulted in no growth delay.

various R3327H tumors shows good vascularization and the absence of large areas of necrosis. Additionally, the responsiveness of this tumor to relatively low doses of radiation suggests that the tumor clonogenic cells might be oxygenated. Studies are in progress to determine whether the R3327H tumors contain a component of hypoxic clonogenic cells or whether the action of misonidazole in this tumor model is unrelated to hypoxia.

The preliminary studies performed with cisplatin, vincristine, etoposide, and estramustine phosphate indicate that the rate of tumor progression can be slowed by the administration of these drugs. Complete control of this rat tumor has not been achieved in any chemotherapeutic protocol tested to date. Cisplatin had the greatest effect on slowing tumor regrowth, whereas vincristine and etoposide had a lesser effect. The drug regimen used for the administration of estramustine phosphate resulted in approximately 27% loss of body weight, and the slowing of tumor growth observed may have resulted because of the compromised nutritional status of these animals (13,14). Block et al (15,16) have shown that a variety of chemotherapeutic agents including 5-FU, cyclophosphamide, doxorubicin, cisplatin, and methotrexate have some effect on the R3327G tumor. These preliminary studies confirm a small degree of antitumor activity by cisplatin on this animal tumor. In addition, the effectiveness of vincristine and etoposide suggest that these agents could play a role in the management of human prostatic cancer.

Estramustine phosphate is presently undergoing clinical trials under the direction of the National Prostatic Cancer Project. In a detailed study with the R3327H tumor, Muntzing et al (17) reported a marked tumor regression with dosages of 100 mg/kg 3 days/week for 4 weeks. These investigators documented a 30% weight loss in the treated animals. In our studies, the tumor growth inhibition observed with this drug was transient and paralleled the severe weight loss induced in the animal, suggesting that a negative nutritional factor instead of a direct action of the drug could be responsible for the observed tumor response. Additional studies are required to distinguish between these two effects.

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Toxicity of Aziridinylbenzoquinone Administered Iv to Beagle Dogs[1,2]

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The toxicologic effects of aziridinylbenzoquinone (AZQ) were evaluated in beagle dogs given the drug iv using several treatment schedules. AZQ was lethal to dogs at 1.25 mg/kg (25 mg/m^2) when administered as a single iv injection and at 0.31 mg/kg/day (6.2 mg/m^2/day) when administered as five daily iv injections. The lethal dose of AZQ in dogs treated with a single iv injection per week repeated for 6 consecutive weeks was 0.63 mg/kg/week (12.6 mg/m^2/week). When administered as three courses of five daily iv injections interspersed with 9 days' rest, AZQ was lethal to dogs at a dose of 0.16 mg/kg/day (3.2 mg/m^2/day). The most prominent sites of AZQ toxicity were lymphoid tissue, bone marrow, and gastrointestinal tract. Lymphoid toxicity was characterized by lymphoid depletion of spleen, thymus, lymph node, and tonsil. Bone marrow toxicity was manifested by hematologic changes (including leukopenia, thrombocytopenia, and anemia) and by hypocellularity of the bone marrow. Gastrointestinal toxicity included clinical signs (such as anorexia, emesis, diarrhea, and body weight loss) and histopathologic lesions (ie, degeneration and depletion of the intestinal crypt epithelium). Less consistent sites of AZQ toxicity were liver (characterized by increased SGPT, SGOT, and alkaline phosphatase levels and by hepatic centrilobular congestion) and kidney (manifested by elevated serum BUN). Although AZQ is highly lipid-soluble and has significant antitumor activity in intracerebrally implanted experimental tumors, no dog given AZQ developed treatment-related lesions in the brain. AZQ, a compound having significant antitumor activity in several experimental tumor systems, produced toxic effects characteristic of many anticancer drugs; the effects appeared reversible upon cessation of AZQ administration. [Cancer Treat Rep 66:1845–1851, 1982]

Doxorubicin, daunorubicin, and mitomycin are natural products with significant antitumor activity (1–4). A common chemical characteristic of these three natural products is the presence of a quinone structure. Of the several hundred purely synthetic quinones thus far screened for antitumor activity by the National Cancer Institute (NCI), Bethesda, MD, only the *p*-benzoquinone derivatives containing multiple aziridinyl groups have demonstrated antitumor activity (5).

Although doxorubicin and daunorubicin are classified as intercalating agents (6), Lin et al (7) have proposed that quinones can also undergo reduction in hypoxic cells, resulting in the production of an alkylating agent. Since the aziridinylquinones are highly lipid-soluble and possess a low degree of ionization, two requirements for CNS penetration as suggested by Rall and Zubrod (8), several of these compounds have been tested for activity against intracerebrally transplanted L1210 murine leukemia cells and against ependymoblastoma of the brain. The aziridinylbenzoquinones have significant activity in both of these tumor systems (9) and may prove to be important in the treatment of human tumors involving the CNS. The present study describes the clinical and pathologic changes in beagle dogs given aziridinylbenzoquinone (AZQ).

MATERIALS AND METHODS

Male and female beagle dogs (7–12 months of age) were supplied by Laboratory Research Enterprises (Kalamazoo, MI). The dogs were housed in individual stainless steel cages in rooms having a controlled tem-

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perature (21°C ± 2°C), relative humidity (50% ± 5%), and photoperiod (0600–1800 hours, lighted period). Each dog was given 300 g of dog chow once daily for a 1-hour feeding period; water was provided ad libitum.

Prior to treatment, the dogs were bled at least three times at 3–4-day intervals to determine pretreatment hematologic and clinical chemistry values. Hematologic tests included the hematocrit by microtube centrifugation, hemoglobin by the cyanmethemoglobin procedure, erythrocyte and platelet counts using a Coulter counter, reticulocytes by methylene blue stain (reported as percent of erythrocytes), and differential leukocyte count by Wright's stain. Clinical chemistry parameters included BUN by the urease method; fasting blood glucose by the hexokinase method; alkaline phosphatase (AP) by the hydrolysis of *p*-nitrophenylphosphate; SGOT and SGPT by the enzymatic oxidation of nicotinamide adenine dinucleotide; creatinine by the reaction with sodium picrate; total bilirubin by the reaction with *p*-benzene diazonium sulfonate; bromsulphalein (BSP) retention by spectrophotometry; and the electrolytes Na^+, K^+, Ca^{++}, and Mg^{++} by atomic absorption and Cl^- by coulometry. For dogs given a single iv injection of AZQ, urine samples were obtained when blood samples were taken. Urine samples were tested for protein, ketones, glucose, and pH with Labstix reagent strips (Ames Co) and examined microscopically for the presence of particulate material.

A necropsy was performed on all dogs, and the following tissues were fixed in 10% neutral buffered Formalin for histopathologic examination: eye, lip, tongue, salivary gland, lymph node (mandibular, regional, bronchial, and mesenteric), tonsil, thymus, thyroid and parathyroid, trachea, esophagus, brain (anterior commissure, optic chiasm, hippocampus, cerebellum, and medulla), pituitary, middle ear, skin, mammary gland, heart, lung, spleen, adrenal, liver, gall bladder, stomach, small intestine (duodenum, jejunum, and ileum), cecum, colon, abdominal aorta, kidney, ureter, urinary bladder, gonad, prostate, uterus, rib with bone marrow, skeletal muscle, and diaphragm.

AZQ (fig 1) was supplied by the Laboratory of Toxicology, NCI, as a clinical formulation and was stored in the dark at 4°C. The 10 mg of AZQ per vial was dissolved in 0.5 ml of sterile dimethylacetamide and then diluted to a final volume of 10 ml with sterile phosphate buffer (0.01 M; pH 6.5) immediately before injection into the saphenous or cephalic vein of the dogs.

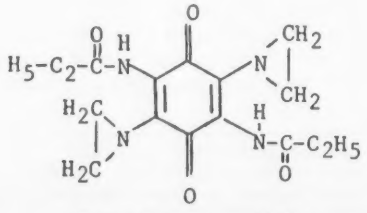

FIGURE 1.—Structure of AZQ.

The protocol used for the studies described in this report was similar to that detailed elsewhere by Prieur et al (10). Basically, dogs were given a single iv injection of AZQ (study 1), five daily iv injections (study 2), three courses of five daily iv injections interspersed with 9 days' rest (study 4), one iv injection per week for 6 consecutive weeks (study 5c), or iv injections for 10 consecutive days (study 5d). Two animals (one male and one female) were treated at each dose level tested.

For studies 1, 2, and 5d, several levels of toxicity were defined according to the protocols described by Prieur et al (10) as modified by the Laboratory of Toxicology, NCI. The defined doses were as follows: *highest nontoxic dose (HNTD)*—the highest dose tested to cause no treatment-related toxic effects; *toxic dose low (TDL)*—the lowest dose tested to cause treatment-related changes in hematologic, clinical, or morphologic parameters, but doubling this dose caused no lethality; *toxic dose high (TDH)*—dose causing treatment-related changes in hematologic, clinical, or morphologic parameters, and doubling this dose resulted in lethality; *lethal dose (LD)*—lowest dose tested to cause treatment-related deaths; and *supralethal dose (SLD)*—twice the lethal dose.

RESULTS

Single-Dose Study

A total of 12 dogs (six males and six females) were treated with a single iv injection of AZQ. One animal from each dose pair was killed on Day 8 and its partner killed on Day 46. When AZQ was administered at either the SLD or LD, the animals were killed when they became moribund. The SLD, LD, TDH, TDL, and HNTD for this treatment regimen are summarized in table 1, while the toxic effects of AZQ administration are summarized in table 2. Treatment-related deaths occurred as late as Day 10 in this study. The acute effects of AZQ administration included gastrointestinal toxicity, pyrexia, and lethargy. Doses of AZQ less than the LD caused less severe and more sporadic signs of toxicity. No treatment-related changes were detected in the urine of any dog administered a single iv injection of AZQ.

AZQ caused pancytopenia, which was most prominent during the first 8 days of the study, in dogs treated with ≥ 0.63 mg/kg (≥ 12.6 mg/m^2). Changes in clinical chemistry values of AZQ-treated dogs were limited to elevated AP and SGPT in dogs given either the LD or TDH of AZQ.

Histopathologic examination of tissues taken from dogs treated with a single iv injection of AZQ revealed

TABLE 1.—Defined doses for AZQ administered iv to dogs

Study No.	Regimen	Defined dose	mg/kg/injection	mg/m²/injection
1	Single iv injection	SLD	2.50	50
		LD	1.25	25
		TDH	0.63	12.6
		TDL	0.16	3.2
		HNTD	0.08	1.6
2	5 daily iv injections	SLD	0.63	12.6
		LD	0.31	6.2
		TDH	0.16	3.2
		TDL	0.08	1.6
		HNTD	0.04	0.8
5d	10 daily iv injections	LD	0.31	6.2
		TDH	0.16	3.2
		TDL	0.04	0.8
		HNTD	0.02	0.4

TABLE 2.—Toxic effects of AZQ administered as a single iv injection to dogs

Dose:	SLD		LD		TDH				TDL		HNTD	
mg/kg:	2.5		1.25		0.63		0.31		0.16		0.08	
mg/m²:	50		25		12.6		6.2		3.2		1.6	
Dog No.:	*481M*	*484F*	*485M*	*480F*	*483M*	*482F*	*487M*	*486F*	*489M*	*488F*	*509M*	*510F*
Day died (D) or killed (K)	6 (D)	5 (D)	8 (K)	10 (D)	8 (K)	46 (K)	8 (K)	46 (K)	46 (K)	8 (K)	46 (K)	8 (K)
Clinical signs												
Decreased food intake	X*	X	X	X		X		X			X	
Body weight loss	X	X	X	X								
Emesis	X	X	X	X								
Diarrhea	X		X			X	X					
Mucus and/or blood in feces		X	X	X			X	X	X	X		
Pyrexia			X	X								
Blood values												
Decreased hematocrit			25† (8)‡	30 (8)	15 (8)	20 (22)						
Decreased hemoglobin				20 (8)								
Decreased erythrocytes		20 (4)	30 (8)	33 (8)	15 (8)	15 (22)		20 (4)				
Decreased leukocytes	40 (4)	88 (4)	90 (8)	80 (8)	70 (8)	80 (8)	40 (8)		40 (8)	25 (4)		
Decreased reticulocytes	95 (4)	95 (4)	95 (8)	95 (8)	95 (8)	95 (8)	95 (4)	95 (4)	90 (4)			
Decreased platelets		40 (4)	80 (8)	33 (8)		33 (8)		27 (4)				
Increased AP			400 (8)	270 (8)		340 (8)						
Increased SGPT			1350 (8)									
Tissue lesions												
Lymphoid depletion												
Spleen	4§	4	3	4								
Thymus	NT	NT	3	3	1							
Lymph node	2	4	2	3	1							
Tonsil	3	4	3	3		1						
Hypocellular bone marrow	4	4	3	3								
Degeneration and/or depletion of small intestine epithelium	2	4	1	1	1							
Centrilobular congestion	1			1								

*X = clinical sign of toxicity was observed.
†% change from the average baseline values of 3 blood samples taken prior to drug administration.
‡Day in study when change was most pronounced.
§Severity of tissue lesion: 1 = mild; 2 = moderate; 3 = marked; 4 = severe; NT = tissue not taken.

lymphoid depletion of the lymph nodes, hypocellularity of the bone marrow, depletion or degeneration of intestinal epithelium, and centrilobular congestion of the liver. Except for a mild lymphoid depletion in dogs given 0.31 mg/kg (6.2 mg/m^2), these lesions were detected only at the LD or SLD of AZQ. No significant histopathologic lesions were observed in tissues taken from dogs killed on Day 46 of the study.

Five Daily Dose Study

A total of 12 dogs (six male and six female) were treated with five daily iv injections of either AZQ or the drug solvent. One animal of each dose pair was killed on Day 6, and the remaining dog was killed on Day 51–53. When AZQ was administered at either the SLD or LD, however, animals were killed either on Day 6 or when the animal became moribund. The defined doses are summarized in table 1, and the toxic effects of AZQ are listed in table 3. Spontaneous deaths occurred as late as Day 11 (6 days after the final AZQ injection) with this treatment regimen. The toxic signs were quite similar to those described for dogs given a single iv injection of AZQ and were limited to dogs treated with either the SLD or the LD of AZQ. The clinical signs of toxicity became evident by Day 4 and increased in severity until the ultimate death of the animal.

Pancytopenia was noted during the first 2 weeks of the study in dogs treated with 0.08 mg/kg/day (1.6 mg/m^2/day). This hematologic alteration was evidently reversible, since all values had returned to near baseline levels by Day 23 of the study.

The major treatment-related tissue lesions were lymphoid depletion of spleen, thymus, lymph node, and tonsil; hypocellularity of the bone marrow; depletion or degeneration of the intestinal crypt epithelium; intestinal hemorrhage; and hepatic centrilobular congestion. These lesions were limited primarily to dogs given the SLD or LD of AZQ.

Multiple-Dose Studies With Periods of Rest Between Treatment

Six dogs were treated with three courses of five daily iv injections of AZQ interspersed with a 9-day rest period between each course of AZQ treatment (study 4).

TABLE 3.—Toxic effects of AZQ administered as 5 daily iv injections

Dose:	SLD		LD		TDH		TDL		HNTD			
mg/kg/day:	0.63		0.31		0.16		0.08		0.04		0.0	
mg/m^2/day:	12.6		6.2		3.2		1.6		0.80		0.0	
Dog No.:	*495M*	*496F*	*493M*	*494F*	*491M*	*492F*	*507M*	*508F*	*525M*	*524F*	*505M*	*506F*
Day died (D) or Killed (K)	6 (D)	11 (K)	11 (D)	6 (K)	6 (K)	52 (K)	6 (K)	53 (K)	6 (K)	51 (K)	6 (K)	53 (K)
Clinical signs												
Decreased food intake	X	X	X	X								
Body weight loss	X	X	X									
Emesis	X		X									
Diarrhea	X											
Blood and/or mucus in feces	X	X	X		X							
Pyrexia	X	X	X									
Lethargy	X											
Blood values												
Decreased hematocrit		30 (9)	20 (9)	25 (6)				15 (9)				
Decreased hemoglobin		27 (9)	15 (9)	18 (6)								
Decreased erythrocytes		28 (9)	25 (9)	18 (6)								
Decreased leukocytes		88 (9)	90 (9)	48 (6)	38 (4)	70 (9)	40 (4)	45 (9)				
Decreased reticulocytes	88 (4)	90 (9)	95 (9)	90 (6)	95 (6)	80 (9)		85 (9)				
Decreased platelets		36 (9)	25 (2)			50(16)						
Increased AP			550 (9)									
Tissue lesions												
Lymphoid depletion												
Spleen	4	3	3	1	1							
Thymus	4	3	3	1								
Lymph node	4	4	3	3	1							
Tonsil	4	NT	3	1	1							
Hypocellular bone marrow	4	4	4	2	2							
Degeneration and/or depletion of intestinal epithelium	4	3	1	1			1					
Centrilobular congestion		1	1									

TABLE 4.—Toxic effects of AZQ administered as 3 courses of 5 daily iv injections with 9 days' rest (study 4) or as weekly iv injections (study 5c)

Study No.:	4		4		4		5c		5c	
Dose: mg/kg/injection:	0.16		0.08		0.04		0.63		0.16	
mg/m²/injection:	3.2		1.6		0/80		12.6		3.2	
Dog No.:	*513M*	*514F*	*511M*	*512F*	*569M*	*568F*	*503M*	*504F*	*499M*	*500F*
Day died (D) or killed (K)	16 (D)	15 (D)	34 (K)	79 (K)	80 (K)	34 (K)	9 (K)	12 (K)	37 (K)	81 (K)
Clinical signs										
Decreased food intake	X	X					X	X	X	X
Body weight loss	X	X		X			X	X		X
Emesis	X						X	X		X
Diarrhea	X	X		X			X	X		
Blood in feces	X	X		X	X		X	X		
Pyrexia	X	X					X	X		
Blood values										
Decreased hematocrit	20 (15)	25 (11)		15 (15)		29 (16)		28 (9)		
Decreased hemoglobin	14 (16)	25 (11)		15 (15)		27 (16)		15 (9)		
Decreased erythrocytes	25 (16)	30 (11)		15 (15)		30 (16)		28 (9)		
Decreased leukocytes	90 (11)	88 (11)	40 (30)	45 (30)		46 (16)	90 (9)	90 (9)	60 (18)	40 (18)
Decreased reticulocytes	95 (9)	95 (11)	65 (23)	57 (25)	95 (16)	95 (4)	80 (4)	90 (11)	80 (18)	80 (18)
Decreased platelets	77 (15)	55 (11)	50 (29)	33 (30)	75 (16)		67 (9)	70 (8)	20 (23)	
Decreased glucose							30 (9)	30 (11)		
Increased BUN	400 (16)						475 (9)	200 (11)		
Increased BSP retention	520 (15)									
Increased AP	520 (16)	60 (11)					350 (9)	375 (11)		
Increased SGPT									390 (16)	
Tissue lesions										
Lymphoid depletion										
Spleen	2	2					2	2		
Thymus	NT	4					2	4		
Lymph node	3	2					2	2		
Tonsil	3	3					2	2		
Hypocellular bone marrow	2	4					3	3		
Degeneration and/or depletion of intestinal epithelium	2	2	1	2	1	1	3	2		
Centrilobular congestion	2	1						1		

The dose levels used in this study were the TDH, TDL, and HNTD as defined for study 2. Only the TDH dose (0.16 mg/kg/day; 3.2 mg/m²/day) caused significant signs of toxicity, first observed on Day 11 and increasing in severity until the eventual death of the animal (table 4). Pancytopenia was observed in both dogs treated with 0.16 mg/kg/day, with less severe and more sporadic changes in dogs given lesser doses of AZQ. Elevated BUN, BSP retention, and AP were noted in one dog administered 0.16 mg AZQ/kg/day. No other clinical chemistry changes were detected in dogs treated with this dosing regimen. As with the other treatment schedules, lymphoid depletion, bone marrow hypocellularity, intestinal degeneration, and hepatic centrilobular congestion were characteristic of AZQ-induced tissue lesions. These histopathologic alterations were prominent only in dogs given 0.16 mg/kg/day. Although elevated BUN levels were sporadically detected in animals administered AZQ, no treatment-related lesions were observed in the kidneys of any animal treated with AZQ.

Four dogs were treated with a single iv injection of either 0.63 or 0.16 mg/kg/week (12.6 or 3.2 mg/m²/week) for up to 6 consecutive weeks (study 5c). The doses chosen for this study were the TDH and TDL as defined for study 1. Both dogs given 0.63 mg/kg/week died after receiving two courses of AZQ treatment. The toxic signs, similar to those described in previous studies, were first evident on Day 8 and became progressively worse until the eventual death of the animal (table 4). Hematologic and clinical chemistry changes were detected primarily in dogs treated with 0.63 mg AZQ/kg/week and included pancytopenia, elevated levels of BUN and AP, and decreased blood glucose. The histopathologic lesions noted in the tissues of those dogs were typical of the lesions described previously for AZQ.

When administered as ten daily iv injections (study 5d), the defined doses of TDL and HNTD for AZQ were one-half the amount (in mg/kg/day) of those given as five daily injections (table 1). As shown in table 5, the toxic signs were similar to those described for other treatment schedules and were observed in dogs given either 0.31 or 0.16 mg of AZQ/kg/day (6.2 or 3.2 mg/

TABLE 5.—Toxic effects of AZQ administered as 10 daily iv injections

Dose: mg/kg/day:	0.31		0.16		0.08		0.04		0.02	
mg/m²/day:	6.2		3.2		1.6		0.8		0.4	
Dog No.:	*521M*	*516F*	*517M*	*520F*	*519M*	*518F*	*523M*	*522F*	*571M*	*570F*
Day died (D) or killed (K)	11 (K)	12 (D)	11 (K)	58 (K)	11 (K)	58 (K)	11 (K)	57 (K)	11 (K)	58 (K)
Clinical signs										
Decreased food intake	X	X	X	X						X
Body weight loss	X	X	X	X	X	X	X	X		
Emesis	X									
Diarrhea				X						
Blood and/or mucus in feces				X						
Pyrexia	X	X								
Blood values										
Decreased hematocrit	15 (11)	15 (11)		18 (11)						
Decreased hemoglobin	30 (11)			20 (11)						
Decreased erythrocytes	15 (11)	23 (11)		20 (11)						
Decreased leukocytes	95 (11)	97 (11)	76 (11)	70 (11)	40 (11)	50 (11)	23 (6)	40 (11)		
Decreased reticulocytes	100 (11)	95 (11)	95 (11)	95 (11)	60 (7)	95 (11)				
Decreased platelets	99 (11)	92 (11)	18 (11)	20 (11)	45 (4)					
Increased BUN	160 (11)									
Increased BSP retention	500 (11)									
Increased AP	660 (11)	700 (11)		160 (18)						
Increased SGOT	35 (11)	30 (11)		40 (2)						
Increased SGPT		40 (11)		30 (11)						
Tissue lesions										
Lymphoid depletion										
Spleen	3	3	1							
Thymus	3	3	2		1					
Lymph node	3	3	1		1					
Tonsil	4	3	1							
Hypocellular bone marrow	4	4	4							
Degeneration and/or depletion of intestinal epithelium	2	2	1		1		1			
Centrilobular congestion	1	1								

m²/day). Clinical signs of toxicity were first noted on Day 10 in dogs receiving 0.31 mg of AZQ/kg/day, and by Day 12, both dogs were dead. Hematologic, clinical chemistry, and histopathologic changes associated with this dosing regimen were similar to those previously described for all other regimens.

DISCUSSION

The defined doses of LD, TDH, TDL, and HNTD for AZQ given iv to dogs following various dose regimens are summarized in table 1. As indicated by studies 1 and 2, AZQ was approximately equitoxic whether given as a single iv injection or as five daily iv injections, when total dose administered was compared. The toxicity of AZQ was apparently increased, however, by two of the treatment schedules tested. The TDH of study 2 was lethal to dogs when a 9-day rest period was interposed between each series of five daily doses (study 4). Similarly, the TDH of study 1 was lethal to dogs when given as single doses at weekly intervals (study 5c). This increased toxicity by the divided dosing regimen was not totally unexpected, however, since the most significant changes brought about by AZQ treatment were somewhat delayed in onset, first appearing 6–15 days after the initial dose of AZQ. Thus, if a second course of AZQ treatment were begun during this time, it is conceivable that the toxicity of AZQ would be increased.

Regardless of the dosing regimen, the most prominent toxic effects produced by iv treatment with AZQ occurred in the lymphoid tissue, bone marrow, and intestine. More sporadic and less severe toxic effects were also detected in the liver and kidney of the dogs. The incidence and severity of these lesions were directly related to the dose of AZQ given and were limited primarily to animals given the TDH or greater dose. Regardless of the dosing regimen, the toxic effects of AZQ were apparently reversible upon cessation of drug administration.

Lymphotoxicity was manifested primarily as lymphoid depletion of the lymph node, spleen, tonsil, and thymus. Other treatment-related lymphoid changes included

hemorrhage in the lymph nodes and ulceration of the tonsil.

The toxic effects of AZQ on the bone marrow were characterized by alterations in the hematologic values and by hypocellularity. Severe leukopenia, thrombocytopenia, and reticulocytopenia were observed in dogs treated with the LD of AZQ. Less severe, but still quite prominent, decreases in leukocyte and reticulocyte counts were also noted at the TDH dose level. The bone marrow was mildly to severely hypocellular in all animals given the TDH or greater dose of AZQ and necropsied prior to the last scheduled necropsy date for a given study. Since the myeloid/erythroid ratios were not altered in any of these animals, AZQ apparently had no specific effect on either the myelogenous or erythrocytic series of cells.

The gastrointestinal toxicity of AZQ was indicated by signs such as decreased food intake; emesis; diarrhea; and blood, bile, and/or mucus in the feces. Histopathologic examination of the gastrointestinal tract revealed depletion and degeneration of crypt epithelium.

Results of liver function tests, including elevated AP, SGOT, SGPT, and/or BSP retention time, indicated that hepatic damage had occurred following AZQ administration. These changes were observed most often during the first 10 days following administration of AZQ at the TDH or greater dose level. Histopathologic examination revealed centrilobular congestion in the livers of several dogs. Although renal toxicity was not detected by histopathology, there was clinical chemistry evidence of renal damage, in that several animals given the TDH or greater dose of AZQ developed elevated serum BUN.

In summary, AZQ produced significant toxic effects to the lymphoid tissues, bone marrow, intestine, kidney, and liver. The toxic effects of AZQ were similar for all treatment schedules tested. The changes occurred primarily in animals given the TDH or greater dose of AZQ and were reversible in those dogs that survived the entire recovery period. In addition, there appeared to be a rather prolonged latency period of up to 10 days between the initiation of AZQ treatment and the development of severe toxic changes, which may explain the increased AZQ toxicity observed when the drug was given on an intermittent basis. It is of interest that AZQ, which has the chemical characteristics required for good CNS penetration, caused no detectable changes in the CNS at less than lethal doses of the drug.

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3-Deazaguanine, a Candidate Drug for the Chemotherapy of Breast Carcinomas?[1,2]

Tasneem A. Khwaja[3,4,*]

3-Deazaguanine (3-DG) is a new purine antagonist that is active against slow- and rapid-growing solid experimental tumors, especially those that are models for human breast carcinomas. These tumors include mammary adenocarcinomas 13762, R3230AC, and C3H/16C. 3-DG also showed positive activity against leukemias L1210 and L1210/araC, adenocarcinoma 755, and EMT-6 mammary adenocarcinoma. On the basis of its activity against these tumors, 3-DG is to undergo clinical trials. [Cancer Treat Rep 66:1853–1858, 1982]

3-Deazaguanine (3-DG) is a structural modification of guanine in which nitrogen in the 3-position of the purine ring has been replaced with a carbon atom and a hydrogen atom (fig 1). The synthesis of 3-DG was accomplished by Cook et al (1), who also described its potent in vitro antiviral activity against both DNA and RNA viruses (2). The antitumor activity and biochemical pharmacology of 3-DG have been briefly reported (3–6). In this report the details of the antitumor activity of 3-DG are presented.

MATERIALS AND METHODS

Sources of animals and tumors.—The animals were obtained through Mr. Clarence R. Reeder, Animal Genetics and Production Branch, Developmental Therapeutics Program, National Cancer Institute, Bethesda, MD. The tumor lines were obtained from Southern Research Institute, Birmingham, AL (leukemias L1210, L1210/araC, and L1210/6MP; adenocarcinoma 755; mammary adenocarcinoma C3H/16C; colon adenocarcinoma 38; Lewis lung carcinoma; and B16 melanotic melanoma); Dr. Russel Hilf, University of Rochester, Rochester, NY (mammary adenocarcinoma R3230AC); Mason Research Institute, Worcester, MA (mammary adenocarcinoma 13762), Arthur D. Little, Inc, Boston, MA (L1210 XII/FU); and Dr. Sarah Rockwell, Yale University, New Haven, CT (EMT-6 mammary adenocarcinoma).

Maintenance of animals and tumors.—These tumors were maintained by serial transplantation in appropriate hosts (or when not in use were kept frozen in a liquid nitrogen freezer) in the Animal Tumor Research Facility of the Los Angeles County-University of Southern California Comprehensive Cancer Center. The animals in each shipment were quarantined, and the acceptable animals were kept in holding rooms until their weights were appropriate (mice, 18–22 g; rats, 70–90 g) for tumor transplantation or experimentation. Tumor transplantations were carried out in separate clean laboratories using standard protocols established by the Drug Evaluation Branch of the National Cancer Institute. Leukemia L1210 was maintained by ip inoculation of the ascites cells (1×10^5) in DBA/2 mice. Leukemia L1210/araC was maintained by weekly ip transplants of 1×10^5 cells in BDF_1 (C57BL/6 × DBA/2) female mice. Leukemias L1210/6MP and L1210/FU

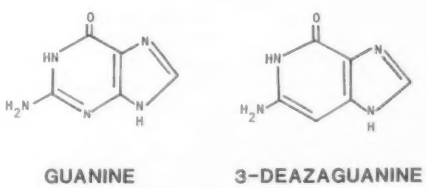

FIGURE 1.—Structures of guanine and 3-DG.

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were maintained by weekly serial transplantation of 1 × 10^6 cells in DBA/2 female mice. Resistance to 6-mercaptopurine (6-MP) was maintained by five daily sc injections of 6-MP (10 mg/kg) beginning 24 hours after transplant. L1210 XII (L1210/FU) mice were treated with seven daily ip injections of 5-FU at a dose of 20 mg/kg of body weight. Adenocarcinoma 755, B16 melanoma, Lewis lung carcinoma, and colon adenocarcinoma 38 were maintained by serial tumor (2–4-mm^3 pieces) transplants (sc in left axillary region) in C57BL/6 female mice. Similarly, mammary adenocarcinomas R3230AC and 13762 were maintained in Fischer 366 female rats. EMT-6 mammary adenocarcinoma was maintained in suspension culture in Eagle's minimum essential medium containing 10% fetal calf serum; each animal ($CD2F_1$ female) received 2 × 10^6 cells sc for experimentation. Lung-passed mammary adenocarcinoma C3H/16C was maintained in C3H female mice according to the method of Corbett et al (7).

Drugs and drug preparations.—3-DG was obtained from ICN Pharmaceuticals, Inc, through the courtesy of Dr. Roland K. Robins. Other standard control drugs were obtained from the NCI Drug Evaluation Branch. For injection purposes, 3-DG was dissolved in sterile 0.2 M phosphate buffer (pH 7.5). 5-FU and 6-MP were dissolved in sterile physiologic saline (pH adjusted to 9.0 with NaOH). All injection solutions were kept at 0°C when not in use. Stability of 3-DG was periodically monitored by high-pressure liquid chromatography.

Test evaluations.—Test evaluations were carried out on the basis of the mean survival time or mean tumor weight of each test group (T) as compared to the control group

TABLE 1.—Effect of 3-DG on growth of L1210 leukemias in BDF_1 female mice

Tumor system	Treatment, ip (Days)	Mean survival (days)	Δ weight on Day 5 (g)	T/C	ILS (%)	No. of survivors at Day 30/No. of mice treated
L1210 (ip) 1×10^5 cells	Saline	8.20	+2.20	—	—	0/10
	3-DG					
	20 mg/kg, qd (1–9)	10.83	+2.00	1.32	32	0/6
	40 mg/kg, qd (1–9)	12.00	+0.40	1.46	46	0/6
	80 mg/kg, qd (1–9)	13.33	+1.70	1.63	63	0/6
	Cytarabine					
	40 mg/kg, qd (1–9)	17.33	−0.20	2.11	111	0/6
L1210/araC (ip) 1×10^5 cells	Saline	8.80	+1.30	—	—	0/10
	3-DG					
	40 mg/kg, qd (1–9)	13.20	−0.67	1.50	50	0/6
	80 mg/kg, qd (1–9)	12.20	−1.17	1.39	39	0/6
	Cytarabine					
	40 mg/kg, qd (1–8)	8.20	−0.83	0.93	−7	0/6
L1210/6MP (ip) 1×10^6 cells	Saline	12.63	+1.80	—	—	0/10
	3-DG					
	40 mg/kg, qd (1–9)	12.17	+1.17	0.96	−4	0/6
	80 mg/kg, qd (1–9)	10.67	+0.50	0.84	−16	0/6
	6-MP					
	35 mg/kg, qd (1–9)	12.67	+0.67	1.00	0	0/6
L1210/FU (ip) 1×10^6 cells	Saline	12.33	+2.82	—	—	1/10
	3-DG					
	80 mg/kg, qd (1–9)	13.80	+0.50	1.12	12	0/6
	5-FU					
	25 mg/kg, qd (1–7)	9.33	+0.33	0.76	−24	0/6
L1210 (ic)* 1×10^4 cells	Saline	9.75	+0.17	—	—	0/8
	3-DG					
	40 mg/kg, qd (1–9)	10.67	+0.17	1.09	9	0/6
	80 mg/kg, qd (1–9)	12.83	+0.33	1.32	32	0/6

*ic = intracerebral.

TABLE 2.—Effect of 3-DG on growth of various transplantable solid tumors

Tumor system (sc)	Host	Treatment* (Days)	Tumor weight, in mg	Δ weight on Day 5 (g)	No. of animals with palpable tumors	T/C	Tumor growth inhibition (%)
Adenocarcinoma 755	C57BL/6 mice	Saline	926 (Day 14)	+0.32	8/8	—	—
		3-DG					
		40 mg/kg, qd (1–7)	227 (Day 14)	+0.64	6/6	0.24	76
		80 mg/kg, qd (1–7)	62 (Day 14)	−2.33	2/6	0.07	93
		6-MP					
		35 mg/kg, qd (1–7)	48 (Day 14)	−0.20	2/6	0.05	95
Mammary adenocarcinoma 13762	Fischer rats	Saline	2110 (Day 16)	+8.00	12/12		
		3-DG					
		40 mg/kg, qd (1–9)	800 (Day 16)	+4.00	8/8	0.38	62
		80 mg/kg, qd (1–9)	60 (Day 16)	+4.75	6/8	0.03	97
		Estradiol valerate (sc)					
		14.4 mg/kg, qd (1–7)	2740 (Day 16)	+0.75	4/4	1.3	−30
		5-FU					
		25 mg/kg, qd (1–7)	1560 (Day 16)	−2.00	8/8	0.74	26
Mammary adenocarcinoma R3230AC	Fischer rats	Saline	4540 (Day 21)	+14.8	10/10	—	—
		3-DG					
		20 mg/kg, qd (1–9)	2530 (Day 21)	+11.3	4/4	0.56	44
		40 mg/kg, qd (1–9)	650 (Day 21)	+8.8	2/4	0.14	86
		80 mg/kg, qd (1–9)	100 (Day 21)	+4.8	1/8	0.01	99
		5-FU					
		25 mg/kg, qd (1–7)	1130 (Day 21)	+7.3	2/4	0.25	75
		Estradiol valerate (sc)					
		12.9 mg/kg, qd (1–7)	2620 (Day 21)	−1.0	2/4	0.54	46
EMT-6 mammary adenocarcinoma	$CD2F_1$ mice	Saline	414 (Day 17)	+0.41	8/8	—	—
		3-DG					
		80 mg/kg, qd (3–10)	170 (Day 17)	−0.80	7/8	0.41	59
Colon adenocarcinoma 38	BDF_1 mice	Saline	120 (Day 17)	+0.4	10/10	—	—
		3-DG					
		80 mg/kg, qd (1–9)	80 (Day 17)	−0.67	2/6	0.66	34
		5-FU					
		98 mg/kg, qd (3,10,17)	0	−0.83	0/6	0	100
Lewis lung carcinoma	C57BL/6 mice	Saline	625 (Day 12)	+0.32	8/8	—	—
		3-DG					
		40 mg/kg, qd (1–7)	608 (Day 12)	+0.72	8/8	0.97	3
		80 mg/kg, qd (1–7)	581 (Day 12)	+0.11	8/8	0.93	7
B16 melanoma	C57BL/6 mice	Saline	496 (Day 14)	+0.32	10/10	—	—
		3-DG					
		80 mg/kg, qd (1–9)	940 (Day 14)	−1.00	6/6	1.91	−91
		5-FU					
		20 mg/kg, qd (1–7)	160 (Day 14)	−1.33	6/6	0.32	68

*Unless otherwise specified, injections were given ip.

(C), which received only sterile isotonic saline, and results are expressed as the ratio of T/C obtained from each experiment. Tumor weights (mg) were estimated by measuring length (l) and width (w) of each tumor with a vernier caliper (mm) and using the formula $\frac{l \times (w)^2}{2}$. Any animal weight gain or loss during the first 5 days of each test was recorded. Mortality > 34% in treatment groups in tumor inhibition studies or death within 5 days of initial transplant in survival studies was considered to be due to drug toxicity. Weight loss (eg, > 4 g/animal in mice) was also regarded as a sign of drug toxicity, and antitumor results obtained were not considered. An increase in lifespan (ILS) > 25% in survival time assays for ascites tumors or an inhibition in tumor weight > 58% (solid tumors) was considered significant.

RESULTS

Table 1 shows that 3-DG is active against leukemias L1210 (ILS, 63%) and L1210/araC, the L1210 strain resistant to treatment with cytarabine (ILS, 50%). 3-DG was not active against L1210/6MP or L1210/FU, the L1210 strains that are resistant to 6-MP and 5-FU treatment, respectively. In solid tumor systems (table 2), 3-DG almost completely inhibited the growth of adenocarcinoma 755, mammary adenocarcinoma 13762, and mammary adenocarcinoma R3230AC, resulting in tumor weight inhibition of 93%, 97%, and 98%, respectively. 3-DG was very effective in inhibiting the growth of C3H mammary adenocarcinoma 16/C (table 3 and fig 2) but showed marginal activity against EMT-6 mammary adenocarcinoma and colon adenocarcinoma 38 (table 2). 3-DG was inactive in Lewis lung carcinoma and B16 melanoma systems (table 2). 3-DG was also tested for its activity against intracerebral implants of leukemia L1210 (1×10^4 cells/mouse) when the drug was administered ip. Our studies show (table 1) that 3-DG was active in this system (ILS, 32%).

DISCUSSION

3-DG in experimental animal leukemia models (table 1) exhibits acceptable activity against leukemia L1210 and cytarabine-resistant leukemia L1210 lacking deoxycytidine kinase. 3-DG was not active against L1210/6MP leukemia lacking hypoxanthineguanine phosphoribosyl transferase and L1210/FU lacking a pyrimidine phosphoribosyl transferase, suggesting that the drug needs to be phosphorylated to be effective (4). In solid tumor systems (tables 2 and 3), 3-DG treatments almost completely inhibited the growth of adenocarcinoma 755, mammary adenocarcinoma 13762, and mammary adenocarcinoma R3230AC. These results are extremely

TABLE 3.—Effect of 3-DG on growth of 16/C mammary adenocarcinoma in $B6C3F_1$ female mice*

Treatment, ip (Days)	Median survival time (days)	% ILS	Duration of remission: T – C value (days)	Activity rating†
Saline	48	—	—	—
3-DG				
40 mg/kg, qd (1–9)	50 (42.5)†	4 (31)†	4.0 (9.5)†	+ (+ +)
80 mg/kg, qd (1–9)	65.5	36	7.5	+ +
5-FU				
20 mg/kg, qd (1–9)	53	10	2.5	+
Saline	34	—	—	—
3-DG				
400 mg/kg, qd (3,7,10,14,17)	62	82	10.2	+ +
5-FU				
98 mg/kg, qd (3,7,14)	62	82	15.0	+ +
Saline	29.5	—	—	—
3-DG				
400 mg/kg, qd (3,10,17,24)	68.0	130	7.0	+ +
400 mg/kg, qd (3,7,10,14,17,21,24)	74.0	150	22.0	+ + + +
5-FU				
98 mg/kg, qd (3,10,17,24)	55.5	88	17.0	+ + +

*Groups of 6–8 mice were given sc transplants of 1–2-mm^3 fragments of tumor on Day 0. Treatments were started 24 hrs or 3 days after implant. T – C value represents tumor growth delay and is based on time required by treatment groups to reach 750 mg in weight minus time required by controls to reach same weight.
†Ref. 7.

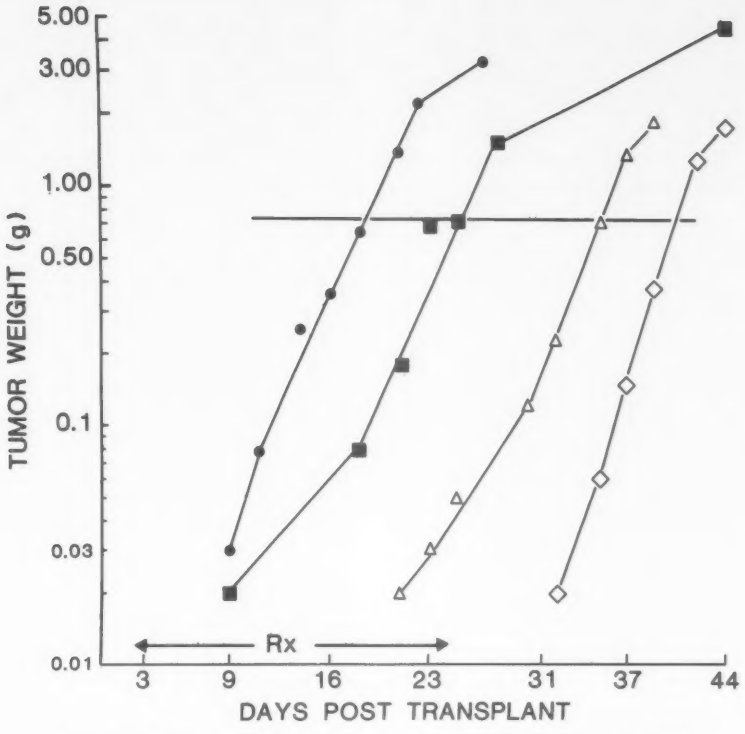

FIGURE 2.—Effect of 3-DG on growth of C3H/16C mammary adenocarcinoma in $B6C3F_1$ female mice. ● = saline controls; ■ = 3-DG, 400 mg/kg, Days 3, 10, 17, and 24; Δ = 5-FU, 98 mg/kg, Days 3, 10, 17, and 24; and ◇ = 3-DG, 400 mg/kg, Days 3, 7, 10, 14, 17, 21, and 24.

interesting in the sense that mammary adenocarcinoma R3230AC is a non-hormone-dependent, estrogen-sensitive, slow-growing tumor, and a model for postoperative breast carcinomas. Mammary adenocarcinoma 13762 is a rapidly growing non-hormone-dependent tumor that is not responsive to estrogen treatment (8). Both of these tumors are differentiated and, under hormonal stimulation, show milk-like secretions (8–10). They represent models analogous to different types of human breast cancers and differ in their responsiveness to treatment with conventional chemotherapeutic agents (8–12). 3-DG was also evaluated against C3H mouse mammary adenocarcinoma 16/C (table 3 and fig 2). This is a highly metastatic tumor with a tumor-doubling time of 2–3 days and responds to most of the agents reported to be active against breast cancer in women, eg, doxorubicin, cyclophosphamide, 5-FU, and melphalan (7). Drugs like 6-thioguanine or 6-thioguanine-2′-deoxyriboside were not active against this tumor (7). 3-DG at a dose of 400 mg/kg twice weekly completely inhibited the growth of this tumor, resulting in prolonged remission (22 days) and a significant ILS (150%) in these animals. The results were comparable to or better than those obtained with 5-FU. Corbett et al (7) have reported positive activity for 3-DG in C3H mammary adenocarcinomas 16/C and 13/C at doses of 43 mg/kg/day for 9 days, confirming our results. 3-DG treatment did not produce significant inhibition of the growth of a slow-growing murine colon adenocarcinoma 38 (table 2). In all of the experiments described above, no apparent signs of drug toxicity, such as gross weight loss, diarrhea, or hair loss, were observed at the end of treatments at therapeutic dosages, eliminating any possibility that the tumor growth inhibition may be due to toxic effects on the hosts. 3-DG was ineffective in the treatment of mice bearing Lewis lung carcinoma or B16 melanoma transplants (table 2). It is interesting to note that 3-DG is a potent inhibitor of estrogen receptor-positive mammary adenocarcinoma R3230AC, whereas it stimulated the growth of B16 melanotic melanoma, which also has estrogen receptor in its cytosol and is responsive to antiestrogen therapy (13).

Our studies demonstrate that 3-DG is very active in inhibiting the growth of slow- and rapid-growing solid tumors, especially those that are models for human breast carcinomas. The marginal or total lack of activity of 3-DG in other experimental tumor systems suggests that, unlike common chemotherapeutic agents, 3-DG may not act like a general cytotoxic agent. In view of these data and the data of the National Cancer Institute showing significant inhibition of the MX-1 mammary human tumor xenograft implanted beneath the renal capsule, 3-DG has been approved at the DN2A level by the Decision Network of the National Cancer Institute for development to clinical trial.

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Primary Non-Hodgkin's Lymphoma of Bone: Results in 15 Patients Treated by Radiotherapy Combined With Systemic Chemotherapy[1]

Gaetano Bacci,[2] Piero Picci,[2,*] Franco Bertoni,[3] Franco Gherlinzoni,[2] Pierina Calderoni,[2] and Mario Campanacci[2,4]

The authors report on the results obtained in 15 patients with primary non-Hodgkin's lymphoma (NHL) of bone treated by radiotherapy and polychemotherapy; 86% of the patients (13 of 15) were continuously disease-free at a median follow-up of 70 months (42–104). No local recurrence was observed. The necessity of clearly distinguishing primary non-Hodgkin's lymphoma of bone from Ewing's sarcoma is discussed. [Cancer Treat Rep 66:1859–1862, 1982]

Primary non-Hodgkin's lymphoma (NHL) of bone is a rare extranodal lymphoma histologically identical to that arising in lymphoid or soft tissue but presenting initially as a localized solitary bone lesion. This tumor has a significantly different clinical course and a much better prognosis than NHL secondarily affecting bone. Usually, primary NHL of bone is readily distinguished from classic Ewing's sarcoma, even if occasionally a sound histologic diagnosis may be difficult because of features that are intermediate to the two types. The difference in prognosis between these two forms makes the distinction more than academic.

Because the response to radiation is rapid and complete, this modality is preferred by most authors for the local control of primary NHL of bone. In the last decade, on the basis of the results of combined therapy in Ewing's sarcoma, some authors (1,2) have suggested combination chemotherapy even in those cases of NHL of bone where no regional or distant spread of disease is evident.

The purpose of this paper is to report the results obtained in 15 consecutive patients with primary NHL of bone treated at the Bone Tumor Center of the Istituto Ortopedico Rizzoli between 1972 and 1978 by radiotherapy combined with systemic adjuvant polychemotherapy.

MATERIALS AND METHODS

Between 1972 and 1978, 142 patients with histologically confirmed malignant round cell tumors were seen at our center; 96 had Ewing's tumor (16 metastatic and 80 localized), 11 had transitional forms (four metastatic and seven localized), and 35 had NHL of bone (20 metastatic and 15 localized). In the present study we consider only the 15 patients with localized primary NHL of bone.

Patients were entered in the study only if, at onset, there was no evidence of other lesions, as determined by extensive clinical findings, radiologic evidence (chest X-rays and tomograms), isotope bone scan, lymphangiographic studies, and bone marrow biopsy. Staging laparotomy was not done in any patient. The clinical features of these 15 patients are summarized in table 1.

Diagnosis was based on the histologic evaluation of representative sections obtained from open biopsy. In addition to standard staining with hematoxylin and eosin, periodic acid-Schiff stain for glycogen and silver stain for reticulin fibers were used to enhance the accuracy of histologic diagnosis. Patients with debatable histology (ie, transitional to Ewing's tumor) were excluded.

The primary tumor was treated by radiotherapy, started 1 week after biopsy, using ^{137}Cs (patients 1–9) or ^{60}Co (patients 10–15). The total dose of radiotherapy varied from 4000 to 4500 rads according to the site and volume of the tumor, patient age, and the presence of severe soft tissue reaction. Opposing field radiation therapy was given when the tumor was located in a long bone; the doses were 3000–3500 rads to the whole bone and an additional 1000–1500 rads to the tumor site. In

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TABLE 1.—Patient characteristics

Patient No.	Age (yrs)	Sex	Bone involved	Histology	Results*	Clinical findings
1	34	M	Femur	Undifferentiated	NED, 104 mos	Bone pain from 2 mos
2	40	M	Femur	Histiocytic	NED, 97 mos	Pathologic fracture
3	41	M	Tibia	Histiocytic	CNS metastasis after 12 mos + 14 mos	Swelling from 1 mo
4	32	F	Ulna	Unclassified	NED, 81 mos	Bone pain from 3 mos
5	50	M	Femur	Histiocytic	CNS metastasis after 10 mos + 12 mos	Bone pain from 6 mos
6	9	M	Tibia	Histiocytic	NED, 74 mos	Bone pain and swelling from 2 mos
7	23	F	L1	Histiocytic	NED, 72 mos	Neurologic symptoms from 1 mo
8	34	M	Femur	Undifferentiated	NED, 70 mos	Pain from 6 mos
9	51	F	Ileum	Histiocytic	NED, 70 mos	Bone pain from 6 mos, swelling from 1 mo
10	18	F	Tibia	Histiocytic	NED, 67 mos	Bone pain and swelling from 3 mos
11	31	M	Ileum	Histiocytic	NED, 65 mos	Pain from 3 mos, anemia
12	21	F	L1	Histiocytic	NED, 65 mos	Neurologic symptoms from 5 mos
13	19	M	Ileum	Histiocytic	NED, 54 mos	Pain from 3 mos
14	52	M	Tibia	Histiocytic	NED, 48 mos	Bone pain from 8 mos
15	36	F	D8	Histiocytic	NED, 42 mos	Bone pain from 3 mos, fever, anemia

*NED = no evidence of disease.

short and flat bones, 3500–4000 rads were delivered to the whole bone, with a boost of 1000 rads to a smaller volume including the tumor itself. In all patients the radiotherapy was delivered over a period of 5–6 weeks (daily dose, 200 rads midplane; 5 fractions/week). Chemotherapy was started on the same day as the biopsy. The initial chemotherapy consisted of vincristine (1 mg/m^2) and cyclophosphamide (500 mg/m^2). Subsequently, adjuvant chemotherapy was given according to the schedule in table 2 and was continued for 2 years. Treatment was delayed if myelosuppression became evident (wbc count < 3000/mm^3 or platelet count < 100,000/mm^3).

All patients had follow-up in our clinic for the detection of recurrent disease, metastasis, and the delayed complications of therapy, every 3 months for 3 years and subsequently every 6 months.

RESULTS

In table 3, the results of this study are reported as well as the results obtained with the same adjuvant

TABLE 2.—Adjuvant chemotherapy scheme (1972–1978)*

	Day 1	Day 2	Day 3	Day 4
Cycle A	ADM (50 mg/m^2)	VCR (1.5 mg/m^2)	CPM (300 mg/m^2)	CPM (300 mg/m^2)
	10 consecutive cycles at 21-day intervals			
Cycle B		VCR (1 mg/m^2) +	CPM (800 mg/m^2)†	
	32 consecutive cycles at 14-day intervals, starting 21 days after end of cycle A			

*ADM = doxorubicin, VCR = vincristine, and CPM = cyclophosphamide.
†Both drugs were given on same day.

TABLE 3.—Primary localized round cell tumor of bone treated between 1972 and 1978*

	RT + chemotherapy			Surgery ± RT + chemotherapy			Total	
	Treated	NED	Local recurrence	Treated	NED	Local recurrence	NED	Local recurrence
Primary non-Hodgkin's lymphoma (15)	15	13 (86%)	—	—	—	—	13 (86%)	—
Ewing's tumor (80)	48	11 (23%)	17 (35%)	32	19 (56%)	4 (12%)	30 (37%)	21 (26%)
P value		< 0.001			—		< 0.001	

*Excluding transitional forms. RT = radiotherapy, NED = no evidence of disease. Unless otherwise indicated, values = No. of patients.

chemotherapy in 80 patients with localized Ewing's tumor treated by us in the same period (3).

All patients with primary NHL of bone who entered the study completed the chemotherapy program except the two who relapsed. During the treatment, both of these patients presented with cerebral metastases and were treated at other institutions with unknown therapy; they died 12 and 14 months after the diagnosis.

The initial tumor response was rapid and satisfactory in all cases. Radiographic changes indicating improvement occurred by 6–8 weeks in all cases. Of the 15 patients, 13 (86%) remained continuously disease-free for 42–104 months ($\bar{x}$ = 70) from the start of therapy. Ten of these patients had follow-up for > 5 years and 12, for > 4 years. The percentage of patients continuously disease-free is by far superior to the percentage observed by us in the 48 patients with Ewing's sarcoma (86% vs 23%) treated in the same period by radiotherapy combined with the identical regimen of adjuvant chemotherapy (table 3).

No local recurrence was observed in our patients with primary NHL of bone. These data contrast with the 35% local recurrence observed in the 48 patients with Ewing's tumor.

Patient tolerance to the chemotherapy was generally good. Only two patients required deviation from the protocol due to pronounced leukopenia or delayed restoration. These two patients were given the entire programmed dose of drugs over a longer period of time. Extrahematologic toxicity was negligible. Despite concurrent use of chemotherapy, toxic effects due to local radiotherapy were moderate; they included edema and induration of the irradiated limb (two patients) and transient infection at the biopsy site (one). No persistent functional impairment or pathologic fracture at the irradiated site was noted over the period considered (3–9 years).

The follow-up time of our patients is still too short to permit evaluation of long-term consequences of the treatment. However, ten patients are alive without any evidence of a secondary tumor at ≥ 5 years from the start of therapy.

DISCUSSION

Today, the treatment of primary NHL of bone varies in different institutions. The accumulated experience with this tumor does not yet allow one to be dogmatic regarding the best treatment. According to some authors (4), when the diagnosis of primary NHL is based on accurate staging including laparotomy the therapy of choice should be radiotherapy only, in view of the potential toxicity of combined chemotherapy. According to other authors (1,5,6), a combination of radiotherapy and chemotherapy is advisable even in those instances in which no regional or distant spread of disease is evident after pathologic staging. Our excellent results with combined therapy and the negligible side effects observed to date have persuaded us to use the latter therapeutic approach.

Although laparotomy has been included in the initial workup at our institute since 1979, we still treat this tumor with combined therapy. However, the chemotherapeutic regimen has been slightly adjusted concerning the duration of treatment (only 12 months) and the number of treatment cycles with doxorubicin (eighty, a total dose of 400 mg/m^2).

Although laparotomy has been included in the initial workup at our institute since 1979, we still treat this tumor with combined therapy. However, the chemotherapeutic regimen has been slightly adjusted concerning the duration of treatment (only 12 months) and the number of treatment cycles with doxorubicin (eight, a total dose of 400 mg/m^2).

1. The overall prognosis of primary NHL of bone is clearly superior to that of Ewing's tumor. This is very important to the performance of a therapeutic trial for Ewing's tumor. For example, if the 15 patients with primary NHL had been included in our trial of adjuvant chemotherapy for localized Ewing's sarcoma, the percentage of patients continuously disease-free, locally treated by radiotherapy alone, would increase from 23% (11 of 48) to 38% (24 of 63).
2. Today, surgical treatment of a primary Ewing's

tumor is being re-evaluated (3,10,11) because (*a*) the use of high-dose radiotherapy (5500–6000 rads) often produces important functional deficit of the limbs, sometimes greater than amputation, and (*b*) there is a high incidence of observed local recurrence after radiotherapy (3,6). On the other hand, according to our results, surgery does not seem to be indicated in the treatment of primary NHL of bone. The radiosensitivity of this tumor permits the delivery of a lower dose of radiotherapy, minimizing local damage. No local recurrence was observed in any of the 15 patients reported, in whom the primary lesion had been treated with only 4000–4500 rads.

3. The site of the localized primary Ewing's tumor is of great importance in determining the prognosis (3,12,13). For example, the prognosis for localized tumor along the skeletal axis is notably worse than for localized tumor in the extremities. On the contrary, in primary NHL the initial site of the tumor does not seem to influence the prognosis. No relapse was observed in our patients with primary localized tumor in the spine or the pelvis.

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Fatal Hepatotoxicity Associated With AMSA Therapy[1,2]

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AMSA is a relatively new chemotherapeutic agent with activity against selected solid tumors and acute leukemia. The usual toxicity from the doses used for solid tumors (120 mg/m^2 every 3 weeks) is mild leukopenia, while at the higher dose levels used for leukemia (50–150 mg/m^2/day for 5 days), there have been reports (1–4) of profound pancytopenia, nausea and vomiting, phlebitis, stomatitis, and hepatic dysfunction. More recently, generalized seizures and cases of ventricular fibrillation temporally related to the AMSA infusion have been observed (5). No case of fatal hepatic toxicity has previously been reported. This case report describes a woman who developed progressive and ultimately fatal liver failure following therapy with AMSA for acute lymphoblastic leukemia.

CASE REPORT

A 44-year-old woman had been diagnosed as having acute lymphoblastic leukemia in March 1975. She achieved an initial remission with prednisone, vincristine, 6-mercaptopurine, and methotrexate and then received central nervous system prophylaxis. Following remission induction, marrow was stored in liquid nitrogen for subsequent autografting. She initially relapsed in July 1979 and was successfully reinduced with L-asparaginase, daunorubicin, vincristine, and prednisone. She subsequently relapsed in January 1980 and was re-treated with prednisone, vincristine, 6-mercaptopurine, and methotrexate without success. In February 1980 she received cyclophosphamide, 1000-rad total-body irradiation, and her autologous remission marrow that was stored 4$^1/_2$ years previously, resulting in a complete remission lasting 10 months. She was then treated with methotrexate and L-asparaginase, but in April 1981 she was again in relapse. The patient was then admitted to the University of Washington Medical Center for experimental therapy with AMSA.

On admission she appeared well with normal vital signs and had no abnormalities during physical examination. Her weight was 57.7 kg. A cbc showed a hematocrit of 34%, a wbc count of 1500/mm^3 (comprised mostly of lymphoblasts), and a platelet count of 18,000/mm^3. Biochemical studies revealed normal serum electrolytes; BUN, 9 mg/100 ml; creatinine, 0.7 mg/100 ml; bilirubin, 0.7 mg/100 ml; SGOT, 25 units/L (normal, 0–25); alkaline phosphatase, 67 units/L (normal, 20–105); amylase, 0.8 units/L (normal, 0–3); and prothrombin time, 100% activity. Chest X-ray and electrocardiogram were normal.

The patient was given AMSA at a dose of 120 mg/m^2 diluted in 500 ml of 5% dextrose in water and infused over 2 hours for 5 consecutive days. She did well for the first 12 days after the initiation of chemotherapy, with only the expected myelosuppression. On Day 13 she became febrile (38.8°C) and started receiving tobramycin and ticarcillin. By Day 16 the fever had abated, and she remained afebrile until 2 days before her death on Day 27. On Day 17 her liver function tests first became abnormal, with a bilirubin of 2.3 mg/100 ml and an alkaline phosphatase of 286 units/L. Over the next 10 days (from Day 17 to Day 27), liver dysfunction progressed to liver failure, which was fatal. The patient's bilirubin steadily increased from 2.3 to 16.5 mg/100 ml; alkaline phosphatase increased to 460 units/L and then plateaued; prothrombin time fell to 15% activity, and SGOT remained normal. A test for hepatitis B antigen was negative. She developed progressive ascites and her weight rose from 58.7 to 69.5 kg despite the use of albumin, furosemide, and spironolactone. On Day 23, the patient developed signs of hepatic encephalopathy,

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with diminished mental status and asterixis. She was treated with oral neomycin and lactulose enemas. Over the next 2 days, the encephalopathy progressed to coma, and for the first time her creatinine began to rise. From Day 25 to Day 27 she remained comatose with increasing renal failure, and she died on Day 27.

An autopsy was performed 8 hours after her death. Findings included a hypoplastic marrow with no evidence of leukemia and evidence of fungal infection with *Candida albicans* with several colonies in the lungs, spleen, pleura, kidney, and liver. The liver weighed 2950 g, was of normal color, and was spotted with several white fibrous nodules. Histologically, the liver lobules were composed of markedly disrupted hepatic cords, with loss of cohesion and radial orientation (fig 1). The enlarged and bizarre hepatocytes lying in the dilated sinusoids had rounded to angulated outlines. Many hepatocytes, especially in the centrilobular zones, had cytoplasmic ballooning with increased amounts of ceroid and hemosiderin pigment. Hepatocyte nuclei were enlarged, were often binucleate, and contained prominent nucleoli. Portal triads were mildly expanded with a lymphocytic infiltrate and bile ductule proliferation. Rare bile thrombi were present in the ductules. The terminal hepatic venules and sublobular veins showed no change of veno-occlusive disease on connective tissue stains. Even allowing for some changes from autolysis, the overall impression was of a diffuse and severe hepatocellular injury.

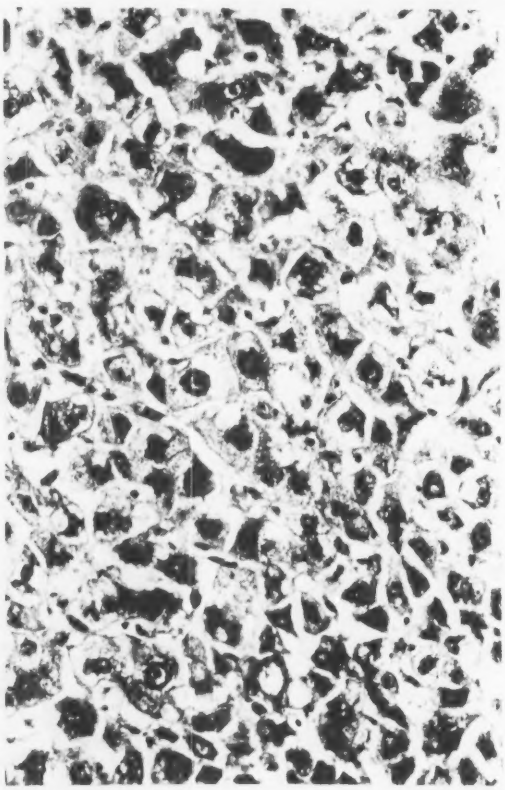

FIGURE 1.—Autopsy section of liver reveals disarray of liver cords with sinusoidal dilation and prominent Kupffer's cells. Hepatocytes have marked anisonucleocytosis, nuclear hyperchromasia with prominent nucleoli, cytoplasmic ballooning with marked ceroid and hemosiderin accumulation, and mild centrilobular cholestasis (Mason trichrome, × 250).

DISCUSSION

Hepatic toxicity associated with AMSA was predicted by the preclinical toxicology studies but was not noted in the phase I trials, in which hematopoietic toxicity was the dose-limiting factor (1,2). However, studies using much higher doses of AMSA for the treatment of acute leukemia demonstrated that AMSA is probably hepatotoxic. Arlin et al (3) reported hepatotoxicity in eight of 31 patients treated with AMSA at a dose of 200 mg/m^2/day for 5 days. As in our case, the evidence for hepatotoxicity was mostly elevations in bilirubin and alkaline phosphatase and was delayed for 2–3 weeks after AMSA was administered. Legha et al (4) reported elevations in bilirubin in 14 of 62 patients treated with AMSA but felt the hyperbilirubinemia may have been due in part to severe infection.

Although it is difficult to define the cause of liver dysfunction in patients with acute leukemia treated with multiple drugs, it seems most likely to us that AMSA was a major cause of the hepatotoxicity that led to this patient's death. The only other medications she received during this hospital course were methadone, ticarcillin, tobramycin, furosemide, spironolactone, docusate, and Tylenol. She had received all of these during other admissions without problems. No structural abnormalities were seen during autopsy. While she had disseminated candidiasis, the extent of the lesions was not sufficient to account for the degree of hepatic dysfunction, nor does it explain the severe hepatocellular injury seen at autopsy. She had received extensive antileukemic treatment prior to receiving AMSA, and this may have made her more susceptible to hepatic damage.

AMSA is an important new antileukemic agent. However, the occurrence of fatal hepatotoxicity in our patient should alert physicians that liver function abnormalities arising during the course of AMSA chemotherapy may be due to this drug and also that AMSA should be used with caution in patients with known hepatic dysfunction.

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Response to Oophorectomy After Tamoxifen Failure in a Premenopausal Patient[1,2]

Alfred M. Kalman, Thomas Thompson, and Charles L. Vogel[3,*]

Previous reports of tamoxifen therapy in premenopausal patients have failed to show responses to ovarian ablation if the patients did not respond to tamoxifen. A case is presented of a response to oophorectomy in a tamoxifen nonresponder and the literature is reviewed. [Cancer Treat Rep 66:1867–1868, 1982]

During the proceedings of a symposium on antiestrogen therapy for hormone-dependent tumors, two groups of investigators (1,2) reported on the use of tamoxifen as primary hormonal therapy in premenopausal patients with metastatic breast cancer. In these two studies, the response to tamoxifen therapy was a strong predictor of a patient's future response to ovarian ablation, with none of 17 tamoxifen failures responding to subsequent ovarian ablation. The present report details a case of a premenopausal woman who failed to respond to tamoxifen therapy, but subsequently had a response to bilateral salpingoophorectomy (BSO).

CASE REPORT

The patient, a 31-year-old menstruating Cuban woman, underwent a left modified radical mastectomy in January 1980 for a $T_3N_{1a}M_o$ poorly differentiated infiltrating ductal carcinoma; 12 of 26 axillary nodes were positive. Estrogen-receptor (ER) assay performed at the time of mastectomy was 102.2 femtomols/mg of cytosol protein and a metastatic work-up was negative. In view of the patient's nodal status, she was entered in a Southeastern Cancer Study Group protocol and randomized to receive 5000 rads of postoperative adjuvant radiotherapy to be followed by six courses of adjuvant cyclophosphamide, methotrexate, and 5-FU (CMF) chemotherapy. The patient was given four courses of CMF and was then lost to follow-up until December 1980 when she had a recurrence with epidural cord compression from bony metastases in the L_1–L_3 region. Restaging workup performed at this time also revealed lytic lesions in the skull, bilateral ilia, upper femora, pubic rami, and right sacrum. A chest X-ray showed a left pleural effusion extending one-third up the left hemithorax. A liver scan was normal and serum alkaline phosphatase was 115 IU/L. The patient was treated with lumbar spine irradiation and started receiving tamoxifen at a dose of 10 mg orally twice a day, with repeat chest X-ray 3 weeks later revealing an increase in the left pleural effusion and no significant change in bone X-rays (pelvis, femora, and skull). The dose of tamoxifen was then increased to 20 mg orally twice a day, and repeat films 8 weeks later showed continued progression of the left pleural effusion so that it now occupied almost the entire left hemithorax. The patient's weight had decreased from 150 to 130 lbs since the start of tamoxifen therapy. Bone X-rays of the skull, pelvis, and femora were again without change. Tamoxifen was discontinued, and the patient was admitted for thoracocentesis and tetracycline sclerosis of her left pleural space. The pleural fluid was noted to be an exudate with negative cytology. Two weeks after tamoxifen was discontinued, a new right pleural effusion was noted on chest X-ray, and the patient underwent sclerosis of her right pleural space followed by a BSO. The fluid from the right pleural space was not sent for diagnostic workup. Four weeks after BSO, a repeat chest X-ray showed the right and left pleural effusions to be unchanged from X-rays taken after tetracycline sclerosis; however, sclerotic repair of the lytic lesions of the ilia, femora, skull, right sacrum, and bilateral pubic rami were noted. Follow-up films during the next 4 months of care revealed continued improvement in all

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lytic lesions mentioned and stabilization of both pleural effusions. Serial determinations of alkaline phosphatase ranged from 109 to 143 IU/L. With objective improvement in the lytic lesions, the patient noticed concomitant decrease in skeletal pain and markedly improved Karnofsky performance status (from 60% before BSO, to 80%). Narcotic analgesic (Empirin #4) requirement was reduced from eight to three tablets daily and the patient's weight stabilized at 130 lbs with no anorexia.

DISCUSSION

A variety of potential hormonal manipulations are available to treat advanced breast cancer. The classical approach to initiating hormonal therapy in ER-positive premenopausal patients with disseminated breast cancer has been surgical ablation of the ovaries. Current preliminary evidence suggests that the antiestrogen tamoxifen may have remisssion-inducing potential and remission duration similar to those of surgical ovarian ablation (3–9). It was therefore elected to initiate hormonal therapy with tamoxifen in our patient. Although we were not able to document a positive cytology from the left pleural effusion, clinically, the patient did not exhibit any signs or symptons to suggest a diagnosis other than malignancy. The marked progression of the left pleural effusion with tamoxifen and the appearance of a new right pleural effusion with tamoxifen withdrawal (10) necessitated institution of a different form of therapy. Even though our patient did not respond to the first hormonal manipulation, a BSO was done and definite objective and symptomatic improvement was noted in the ensuing 4 months.

Previous reports of tamoxifen therapy in premenopausal patients have failed to show responses to ovarian ablation if the patient did not respond to tamoxifen. Our literature review indicates that there have been only 17 patients who have had ovarian ablation after failing tamoxifen; none of these patients responded. However, of the 17 nonresponders, only four were documented to have ER-positive tumors. The remaining patients (eight, ER unknown; four, ER negative; and one, ER borderline) may have had intrinsically hormonally resistant tumors and thus may not have been an ideal population to test the effectiveness of sequential hormonal maneuvers.

Although patients who do not respond to one hormone have a low probability of benefiting from any other endocrine treatment, it has been shown in tamoxifen nonresponders that the subsequent use of androgens or hypophysectomy may yield a response (11). It therefore seems reasonable to assume that a similar change in the hormonal milieu (ie, ovarian ablation) might also be efficacious.

It is of interest to note that while receiving tamoxifen, our patient continued to have regular menstrual cycles, indicating an incomplete antiestrogen effect. Although it is clear that tumor shrinkage by tamoxifen can occur without achieving any irregularity in the menses, one might speculate that patients who manifest evidence of estrogen deprivation while receiving antiestrogen therapy may have a higher rate of response, as suggested by Pritchard et al (2). It is possible that our patient's failure to respond to tamoxifen was secondary to an inability to exert sufficient antiestrogenic effect at the level of the tumor as well as the uterus at the doses prescribed.

Since initial submission of this paper, a study was reported by Hoogstraten (12) also challenging the initial observations of Pritchard et al (2). In Hoogstraten's study, response to tamoxifen therapy did not predict for future response to ovarian ablation because five of 22 premenopausal tamoxifen nonresponders subsequently had a response to BSO, whereas none of 12 tamoxifen responders who underwent BSO at relapse had a response. This study is not strictly comparable to prior studies, however, since tamoxifen was continued after ovarian ablation. Although no generalizations can be derived from our single case report, we still consider oophorectomy as a therapeutic option in premenopausal tamoxifen nonresponders.

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Phase II Evaluation of Bisantrene in Patients With Renal Cell Carcinoma[1,2]

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Bisantrene is a new anthracene bishydrazone derivative thought to function as a DNA intercalating agent. In recently reported phase I studies, responses to this drug were seen in patients with a variety of tumors including renal cell carcinoma (1,2). In addition, in vitro antitumor activity was noted for bisantrene against renal cell carcinoma in the human tumor cloning system. With that assay, in vitro responses (≥ 70% decrease in tumor colony-forming units) were noted in four of 18 patients' tumors growing in culture (3). This background, in addition to the known resistance of this tumor to established chemotherapeutic agents, served as an impetus to conduct a phase II study of bisantrene in patients with renal carcinoma. Our objectives included: (*a*) establishing the efficacy of bisantrene as a single agent used in a single-dose intermittent schedule and (*b*) evaluating the utility of the human tumor cloning system for screening for phase II clinical activity of a new compound.

PATIENTS AND METHODS

Forty-two patients with advanced renal cell carcinoma were entered in this study between August 1980 and September 1981. Histologic confirmation was obtained in 41 patients. An additional patient with typical angiographic findings of a renal hypervascular mass and inferior vena cava tumor thrombus was included. Patients with clearly measurable disease, adequate bone marrow, and adequate hepatic (bilirubin < 2.0 mg/dl) and renal (creatinine < 2.0 mg/dl) functions were selected for the study, regardless of performance status (PS). The presence of an unstable cardiac rhythm or recent congestive heart failure was grounds for exclusion. Bisantrene was administered as an iv infusion in 300–500 ml of 5% dextrose in water over 1–2 hours every 3 weeks. The drug was given either by a peripheral vein or via a Broviac central catheter. Initially, patients received 260 mg/m^2, with subsequent dose increase (10%) or decrease (20%–50%) depending on the absence or presence and severity of toxic effects. Cbcs were determined weekly, and hepatic and renal function tests were obtained prior to each infusion. Later entries (six patients) with adequate marrow reserve received an initial dose of ≥ 280 mg/m^2. In addition, at the invesigators' discretion, patients were pretreated with parenteral Benadryl and/or corticosteroids in attempts to obviate allergic-like reactions during iv infusions of bisantrene.

An adequate trial was defined as two doses of drug with subsequent follow-up. Standard response criteria were applied. A complete response was defined as the disappearance of all clinical evidence of active tumor for at least 4 weeks. A partial response was defined as a ≥ 50% decrease in the sum of the products of two perpendicular diameters of the measured lesions. Patients with liver metastases had to have a 30% reduction in the sums of the liver span measurements below each costal margin. These reductions in tumor or organ size had to last at least 4 weeks, with no simultaneous increase in size or simultaneous appearance of new lesions. Stable disease was defined as a steady state or response less than partial remission maintained for a minimum of 8 weeks. Disease progression was defined as a 25% increase in the size of any measurable lesion or the appearance of new lesions.

The study consisted of 30 males and 12 females, with a median Southwest Oncology Group PS of 1 (range, 0–4). The median age was 64 years (range, 28–84). Nine-

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teen patients had had prior hormonal therapy, four had had prior chemotherapy, and five had had both. Fourteen patients had had no prior systemic therapy. Sites of indicator lesions included lung (24 patients), bone (nine), nodes (eight), soft tissue (five), and liver (four).

RESULTS

Antitumor Activity

Five patients were excluded from response analysis. Two patients died of progressive disease on Days 6 and 12 of their first bisantrene cycle, two were lost to follow-up after the second drug infusion, and one refused further therapy after the first infusion. Two of the 37 evaluable patients had responses. A 51-year-old male (PS = 0) with liver metastases and prior hormonal therapy experienced a partial response lasting 10 weeks, and a 51-year-old female (PS = 0) with lung metastases and prior drug and hormonal therapy was removed from study in partial remission (10+ weeks) after an anaphylactoid reaction. Another patient, a 67-year-old female (PS = 0) with prior hormonal therapy, had near resolution of lung nodules while bone disease progressed after 12 weeks of therapy. Twelve patients had disease stabilization for a minimum of 8 weeks (range, 8–39). Twenty-two patients had clear disease progression during bisantrene therapy.

A total of 169 bisantrene infusions were given. The median dose administered was 280 mg/m^2 (range, 160–370). The median number of infusions per patient was four (range, two to 14). Of the 37 evaluable patients, 28 experienced dose-limiting toxic effects prior to going off study.

Toxicity

Toxicity, assessed in 35 patients, is summarized in table 1. While granulocytopenia (nadir counts determined on Day 8) appeared to be the major dose-limiting effect, there was no evidence that this was cumulative (cumulative dose median, 1040 mg/m^2; range, 520–3860). Further, no clinical cardiac toxicity was evident in this group of patients.

Signs and symptoms comprising an anaphylactoid reaction were seen in six patients; they were abrupt and clinically serious and included hypotension, diaphoresis, shortness of breath, chills, nausea, and vomiting. Other inconstant symptoms in these six episodes were back pain, fever, and a flushing sensation. All six patients had received multiple bisantrene infusions (range, three to 13), and there were no uniform prodromal symptoms during their prior infusions. Further, premedicating five of six of these patients with Benadryl and/or corticosteroids did not prevent the subsequent intrainfusion reaction.

TABLE 1.—Toxicity of bisantrene (35 patients)

Toxic effect	No. of patients (%)
Granulocytopenia (polymorphonuclear neutrophils/mm^3)	
1000–1500	9 (26)
500–999	13 (37)
250–499	5 (14)
0–249	1 (3)
Anemia (Hgb, g/100 ml)	
9.0–9.9	12 (34)
7.0–8.9	8 (23)
Thrombocytopenia (platelets/mm^3)	
75,000–99,999	1 (3)
Nausea/vomiting	12 (34)
Local reactions*	9 (26)
Fever	9 (26)
Chills	5 (14)
Anaphylactoid reaction	6 (17)
Miscellaneous	
Mucositis	1 (3)
Urticaria	1 (3)
Flushing	1 (3)
Diarrhea	1 (3)
Chest pain	1 (3)
Hiccoughs	1 (3)

*Extravasation with subsequent ulceration in 2 patients.

Local reactions were noted in 26% of the patients receiving the drug by peripheral vein. These reactions included pain at injection site without objective findings, discoloration of vein proximally, and local angioedema. Two patients had extravasation with subsequent ulcer formation which healed in 6 weeks. There were no drug-related deaths.

DISCUSSION

Two of 37 evaluable patients (5%) experienced partial remissions during this study, indicating minimal activity of bisantrene against renal cell carcinoma when administered on this schedule. This trial appears adequate in light of 28 of 37 patients experiencing dose-limiting toxic effects and lack of enhanced activity when bisantrene was administered at higher starting doses (≥ 280 mg/m^2) in six patients at the conclusion of the study.

The occurrence of the anaphylactoid reactions may have contributed to this lack of activity. Of the six patients removed from the study subsequent to these anaphylactoid reactions, one had a partial response approaching complete remission, one had stable disease approaching a partial response, and the remainder had stable disease. The etiology of this reaction remains to be defined.

The local reactions noted with bisantrene administration may be related to limited solubility and possible

luminal precipitation of the drug (4). Use of antihistamines and steroids to reduce the incidence of this local phenomenon is being studied in ongoing trials. Central infusions may obviate these problems.

Bisantrene has demonstrated no significant antitumor activity clinically with the schedule and dose utilized. This is disappointing in light of the screening data in the cloning assay where four of 18 patients' tumors showed an in vitro response to the drug. Reasons for this difference between in vitro and in vivo results need to be defined to further assess the usefulness of the assay for in vitro phase II trials.

Despite the low response rate noted in the present study, it is possible that phase II studies using bisantrene in other dosing schedules may reveal antitumor activity against renal cell carcinoma. Such trials are ongoing in other institutions.

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Mitomycin, Hexamethylmelamine, and Vindesine as Second-Line Treatment for Small Cell Cancer of the Lung[1]

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Combination chemotherapy has considerably changed the outlook for patients with small cell lung cancer, and in several reported series there is now a small fraction of long-term survivors. However, 80%–90% of the patients ultimately die of their disease. The results of second-line chemotherapy after relapse are disappointing. Mitomycin, hexamethylmelamine, and vindesine have been reported to be active in the treatment of small cell lung cancer (1–4).

In the present study, 13 male patients with relapsing small cell lung cancer (table 1) were treated with mitomycin (10 mg/m^2 iv on Day 1), hexamethylmelamine (100 mg/day orally on Days 1–14 and 21–36), and vindesine (3 mg/m^2 iv on Days 1, 8, 21, and 28); this cycle was repeated on Day 43.

One patient experienced a partial remission, with dramatic shrinkage of liver metastases lasting for 66 days. Six patients had disease stabilization for a median

TABLE 1.—Patient characteristics, response, survival, and toxicity

Characteristic	No. of patients			
	Response			
	Partial	No change	Disease progression	Total
Median age in yrs (range): 62 (40–70)				
Performance status				
0	0	1	2	3
1	0	1	2	3
2	1	4	2	7
Stage				
Limited disease	0	3	4	7
Extensive disease	1	3	2	6
Previous chemotherapy				
3 drugs	1	2	0	3
4–6 drugs	0	2	4	6
> 6 drugs	0	2	2	4
Overall response	1	6	6	—
Median survival in mos (range): 3.5 (1.5–6.0)				
Hematologic toxicity				
Leukocyte count nadir (cells/mm^3)				
Median (range): 1900 (800–5000)				
No. of patients with < 2000: 7				
Platelet count nadir (cells/mm^3)				
Median (range): 105,000 (40,000–230,000)				
No. of patients with < 75,000: 5				

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of 77 days, and six patients showed disease progression. Of the seven patients with a response or stable disease, two showed further disease progression locoregionally whereas five progressed with liver (three patients) or bone (two patients) metastases. Of the six nonresponders, four progressed with distant metastases (liver, two patients; bone, one; and brain, one), and two progressed locoregionally. Median survival for all patients was 3.5 months (1.5–6.0). The seven patients with a response or stable disease survived for a median of 3.5 months (2.5–4+) and the nonresponders for a median of 3.0 months (1.5–6.0). Hematologic toxicity in these heavily pretreated patients was manageable, with a median leukocyte count nadir of 1900/mm^3 and a median platelet count nadir of 105,000/mm^3 (table 1). One patient showed a transient rise of serum creatinine to 1.69 mg/100 ml. Six patients experienced slight nausea and vomiting. Moderate signs of neurotoxicity with paresthesias and muscle weakness were observed in eight patients.

We conclude that the combination of mitomycin, hexamethylmelamine, and vindesine has acceptable toxicity but no major impact on the natural course of relapsing small cell lung cancer. The activity of mitomycin in small cell lung cancer needs to be further defined.

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Aziridinylbenzoquinone (AZQ) in Advanced Breast Cancer: A Cancer and Leukemia Group B Phase II Trial[1]

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AZQ is a lipophilic aziridinylbenzoquinone that has entered phase II trials (1). Because of evidence of cytolytic activity in human mammary xenografts (2) and murine tumor systems (3), the Cancer and Leukemia Group B (CALGB) initiated a phase II trial in patients with advanced mammary carcinoma.

Female patients with histologically documented breast adenocarcinoma, who were not candidates for surgical resection, radiation therapy, or standard chemotherapy, were studied. No patients had been exposed to more than two chemotherapeutic regimens.

CALGB criteria for entry in the study included measurable disease, ambulatory status (CALGB performance status ≤ 3), total wbc count $\geq 4000/\mu l$, platelet count $\geq 100{,}000/\mu l$, serum bilirubin ≤ 1.5 mg/dl, serum creatinine ≤ 1.5 mg/dl, no prior therapy for 4 weeks before entry, anticipated survival > 2 months, and informed consent.

AZQ was dissolved in 100 ml of normal saline and given to study patients at a dose of 30 mg/m^2 by iv infusion over 30 minutes, once every 3 weeks (1). If there was evidence of minor hematologic toxicity (wbc count of 3500–3999/μl) on the day of re-treatment, the drug dose was reduced by 25%. For more significant toxicity (wbc count of 2500–3499/μl and/or platelet count of 75,000–99,999/μl), the dose was reduced by 50%. For greater toxicity, the drug was withheld, and peripheral blood cell counts were performed at weekly intervals until acceptable for treatment.

Patients had a weekly cbc and platelet count. Repeat serum liver function tests, creatinine, BUN, and glucose were done every 21 days.

Of 41 patients entered, 35 were eligible for study; four did not meet eligibility criteria, and two violated the study design. Eighty-nine percent of the patients had previously failed anthracycline therapy (table 1).

Toxicity was mainly hematologic, causing leukopenia or thrombocytopenia (table 1). Patients became progressively more anemic with repetitive therapy, showing a statistically significant drop in mean hemoglobin

TABLE 1.—Pretreatment characteristics and study results

Characteristics and results	% of patients
Eligible for study: 35 patients	100
Initial performance status	
0	26
1	23
2	48
3	3
Age in yrs	
Mean: 58	
Range: 34–58	
Prior chemotherapy	
1 combination	26
2 combinations	74
No prior anthracycline	11
Dominant site of disease	
Visceral	63
Osseous	17
Soft tissue	20
Cycles of therapy	
Mean No.: 1.6	
> 3	23
Toxic effects	
Anemia (Hgb, 7–9.0 g/dl)	33
Leukopenia (wbcs/μl)	
2000–4000	40
1000–1999	23
< 1000	17
Thrombocytopenia (platelets/μl)	
50,000–100,000	23
20,000–49,999	27
$< 20{,}000$	17
Nausea/vomiting	11
Complete/partial responses: 0	
Stabilization/minor improvement: 7	
Disease progression: 28	

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(11.8 to 10.7 g/dl) by Day 21 of the study.

Twenty-three patients had obvious disease progression. One patient refused evaluation at Day 21, and four died within 2 weeks of the start of the protocol (scored as nonresponders).

The finding of no major antitumor response to this drug in 35 patients precludes a 15% response frequency (with 99% confidence) in pretreated patients with breast cancer.

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Combination Chemotherapy With Vincristine, Carmustine (BCNU), and Bleomycin in Patients With Squamous Cell Bronchial Carcinoma Resistant to Cyclophosphamide, Doxorubicin, Methotrexate, and Procarbazine (CAMP)[1]

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The activity of the CAMP (cyclophosphamide, doxorubicin, methotrexate, and procarbazine) regimen in metastatic non-oat cell bronchial carcinoma (NOBC) was identified by Bitran et al (1) and confirmed by other authors (2,3). However, even for responders, median survival does not exceed 12 months, indicating the need for effective non-cross resistant second-line regimens. Unfortunately, previous treatment reduces the responsiveness to antitumor drugs in NOBC (4). A combination of vincristine (VCR), carmustine (BCNU), and bleomycin (BLM) was prospectively evaluated at our division from July 1979 to December 1980 as second-line chemotherapeutic treatment for patients with advanced NOBC who failed CAMP chemotherapy.

Thirty-two patients with measurable lesions were treated; all of them had squamous cell carcinoma in progression after cobalt 60 radiotherapy to the tumor and regional lymph nodes (45 grays in 15 sessions) and CAMP chemotherapy at the standard dosage described by Bitran et al (1) (median, four cycles). Chemotherapy consisted of VCR (1.2 mg/m^2 iv) and BCNU (100 mg/m^2 iv) given at 0 hours and BLM (5 mg im) given at 6, 30, 54, 78, and 102 hours. VCR and BLM were repeated every 2 weeks; BCNU was repeated every 6 weeks. Chemotherapy was continued until disease progression. Response was evaluated according to standard criteria and was expressed as complete response, partial response, minimal response, unchanged, and disease progression. In 15 patients the indicator lesion was in an irradiated area. Patient characteristics and response to treatment are reported in table 1. Chemotherapy was well-tolerated and treatment was never discontinued because of toxicity.

Our results are similar to those reported by Mintz et al (5), who could not demonstrate any clinical activity of a combination of hexamethylmelamine, BLM, and

TABLE 1.—Characteristics of patients studied

Characteristics	No. of patients
Patients entered in study	32
Patients evaluable	27
Median age: 60 yrs (range, 43–72)	
Sex	
Male	25
Female	2
Median Karnofsky performance status: 75 (range, 40–90)	
Sites of disease	
Locoregional relapse	16
Lymph node metastases	8
Bone metastases	2
Skin metastases	1
Median No. of cycles: 4.5 (range, 1–11)	
Median % of theoretic dose of drugs administered (range)	
VCR: 100 (60–100)	
BCNU: 100 (40–100)	
BLM: 100 (70–130)	
Responses	
Complete	0
Partial	0
Minimal	2
Unchanged	18
Disease progression	7
Sites of disease progression	
Local	6
Lymph nodes	2
Bones	1
Toxicity	
Leukopenia (< 4000 cells/mm^3)	3
Fever	5
Skin pigmentation	1
Pulmonary toxicity at spirometry	3
Mucositis	3
Paresthesias	9
Median survival: 5 mos (range, 1–16)	

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lomustine (CCNU) in CAMP-resistant patients. The use of conventional agents in chemotherapy-refractory bronchial squamous cell carcinoma does not seem to be of clinical utility, and the use of investigational agents appears to be indicated in this situation.

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Leukopenia Associated With Aminoglutethimide Therapy: A Case Report

Several articles within the past year (1,2) have reported on the efficacy and safety of aminoglutethimide compared with surgical adrenalectomy for the treatment of metastatic breast cancer in postmenopausal women. The major side effects of aminoglutethimide include lethargy, a rash, ataxia, and orthostatic dizziness, which rarely necessitate discontinuation of the drug. A search of the literature located only one case report implicating aminoglutethimide as a cause of pancytopenia (3). I report here a second case of severe neutropenia related to the use of this drug.

The patient, a 69-year-old woman, presented to another hospital in December 1980 with a pathologic fracture of the right femur. Biopsy of this site as well as of a fungating tumor of the right breast revealed infiltrating ductal carcinoma. Estrogen and progesterone receptors were strongly positive. X-rays revealed the tumor to be widely metastatic to bone, especially the ribs. The patient then underwent a simple mastectomy of the right breast. The femur was pinned and irradiated to a total dose of 3900 rads. Past medical history revealed a hysterectomy and bilateral oophorectomy in 1939.

Tamoxifen at a dose of 10 mg twice a day orally was started, with symptomatic relief initially. However, by September 1981 the disease had progressed and it was elected to begin aminoglutethimide at a dose of 250 mg twice a day orally and cortisone at a dose of 100 mg orally (60 mg at bedtime and 20 mg twice a day). Two weeks later, the dose of aminoglutethimide was increased to 250 mg four times a day, and the dose of cortisone was reduced to 40 mg orally (20 mg at bedtime and 10 mg twice a day). Cbc at that time revealed a wbc count of 4100/mm^3, with 63 segmented band cells, 30 lymphocytes, four monocytes, one eosinophil, and two basophils. Hemoglobin was 11.3 g/dl and hematocrit was 34%. One month later, the patient returned for routine follow-up, and the cbc revealed a wbc count of 2.3/mm^3, with 60 segmented band cells, 33 lymphocytes, two basophils, three eosinophils, and two band cells. At her second monthly visit 10 weeks after initiation of therapy, the wbc count was 1000/mm^3, with eight segmented band cells, 84 lymphocytes, six monocytes, and two eosinophils. Hemoglobin was 11.8 g/dl and hematocrit was 35%; platelet count was adequate. The patient felt well and had no complaints except for left infrascapular pain, which she had had throughout her disease course but for which she took no medication. A bone marrow biopsy from the left iliac crest revealed new bone growth, myelofibrosis, and metastatic breast carcinoma. Aminoglutethimide was discontinued and cortisone was maintained. When the patient returned the following day with a urinary tract infection and probable gram-negative sepsis, she started receiving cefazolin and gentamicin, but this treatment was changed to ampicillin in 2 days when the culture results were obtained. Her course was complicated by oral thrush, which responded to topical nystatin. The wbc count nadir was 400/mm^3 on the second hospital day, with no granulocytes seen. Seven days after discontinuation of the aminoglutethimide, the wbc count had begun to increase and by Day 12 it had returned to pretreatment levels (3400/mm^3, with two band cells, 25 segmented band cells, 52 lymphocytes, two myelocytes, and three metamyelocytes), where it has remained pending further therapy.

The patient described by Lawrence et al (3) was receiving triamterene for blood pressure control in addition to aminoglutethimide. The bone marrow recovered after discontinuation of the aminoglutethimide, despite continuation of the triamterene. The patient in the present report was receiving no medication except aminoglutethimide and cortisone (50 mg daily). Underlying malignancy and metastatic involvement of the marrow was undoubtedly a major factor in the development of neutropenia in this patient. However, the time course of the fall in wbc counts and the marrow recovery strongly suggests a link with aminoglutethimide administration. In view of this, aminoglutethimide should be used with caution in any patient likely to have a limited bone marrow reserve.

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Intensive Timed Sequential Combination Chemotherapy in Extensive-Stage Small Cell Carcinoma of the Lung

Preliminary evaluation of an intensive timed sequential chemotherapeutic regimen in small cell carcinoma of the lung (SCCL) based on tumor cell kinetics (1) has been presented (2). The kinetic model predicts that following an initial significant tumor kill there will be stimulation of tumor growth (approximately on Days 8–11) at which time the cancer will be particularly susceptible to the effects of chemotherapy. It was our hypothesis that two cycles of this intensive induction regimen followed by local radiotherapy to the primary lesion and by maintenance chemotherapy would increase the complete response rate and prolong survival in patients with extensive-stage SCCL.

Twenty-four previously untreated patients (median age, 59 years) with extensive SCCL were treated with this protocol. A total of 54% of the patients had bone and/or bone marrow involvement with tumor while 25% had liver metastasis. A total of 66% had an Eastern Cooperative Oncology Group performance status of 0, 1, or 2. Patients were treated with CAV (75 mg/kg of cyclophosphamide iv on Day 1, 60 mg/m^2 of doxorubicin iv on Day 8, and 125 mg/m^2 of etoposide iv on Days 9–11). A second cycle of CAV was administered on Day 28. Complete and partial responders received CAVCR (25 mg/kg of cyclophosphamide iv, 40 mg/m^2 of doxorubicin iv, and 1.4 mg/m^2 of vincristine iv) on Day 56. Prophylactic cranial (2100 rads in seven fractions) and local radiotherapy to the lung primary (2000 rads in five fractions) were begun on Day 84 in an effort to decrease the incidence of brain metastasis and local recurrence. Beginning on Day 94, CAVCR was administered at 21-day intervals until relapse. Twenty-two patients were able to receive two cycles of CAV.

Eight of the 24 patients (33%) obtained a complete response (CR) and 12 (50%) obtained a partial response, for an overall response rate of 83%. Median survival for the entire group was 10.8 months, while that for patients attaining a CR was 13 months. The longest survival for patients receiving this treatment regimen was 19 months. All patients have died.

The mean duration of severe leukopenia (< 1000 wbcs/mm^3) during each induction cycle was 13 days, with the wbc count being < 500/mm^3 for a mean of 10 days. A total of 91% of the courses were associated with fever requiring antibiotics. Bacteremia developed during six courses (13%). Platelet transfusions were administered during 15 courses (33%). There were no deaths during the intensive induction therapy.

These results are comparable to our previous experience with extensive-stage SCCL (3) and that reported in the literature (4). We conclude that while intensive induction therapy with this timed sequential chemotherapeutic regimen can be safely administered, we have been unable to demonstrate a significant improvement in CR rate or survival in extensive-stage SCCL through the use of this particular regimen.

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Pulmonary Fibrosis Associated With Lomustine (CCNU): A Case Report

Chemotherapy with cytotoxic drugs is associated with organ-specific toxic effects. Injury to the lungs is very disturbing, since the onset of such damage is insidious. By the time damage is evident on chest X-ray or by pulmonary function studies, it may be irreversible and progressive (1). This may happen despite withdrawal of the inciting agent. Bleomycin and busulfan are well known to cause pulmonary fibrosis (1–3). Reports of lung damage have also been associated with other cytotoxic drugs.

Nitrosoureas, especially carmustine (BCNU) and lomustine (CCNU), are very effective anticancer agents and have been widely used in various solid tumors and hematologic malignancies. Delayed bone marrow suppression has been the dose-limiting toxic effect of these agents in man. Recently, more pulmonary toxicity is being described with carmustine (1,4), and there has been one report with semustine (methyl-CCNU) (5). The patient described in the current report had lung damage from lomustine while being treated for a recurrent glioma. To our knowledge, this is the first report of such damage caused by this nitrosourea.

CASE REPORT

Clinical History

A $7^1/_2$-year-old white male was diagnosed in November 1970 as having a well-differentiated grade I astrocytoma on craniotomy and biopsy. A ventriculoperitoneal shunt was placed, and the child received radiation therapy of 2600 rads to each of three ports in the posterior cranial fossa over a 6-week period. In October 1971, the patient developed marked ataxia, inability to walk, headaches, and vomiting. A computerized tomographic (CT) scan revealed recurrence of his brain tumor, and he started receiving lomustine (130 mg/m^2) every 6 weeks orally. The total dose of lomustine was 1040 mg (1170 mg/m^2) over a period of 26 months (until December 1973). While receiving therapy, the patient had marked subjective and objective improvement in his brain tumor. In March 1972, while he was receiving lomustine therapy, he had one episode of difficulty in breathing, without fever, which cleared without treatment. There were no abnormalities of the chest X-ray at that time. There was no evidence of the tumor on CT scan at the time of discontinuation of therapy. In the first 18 months after discontinuation of therapy, there were three similar episodes of difficulty in breathing, without fever. With only one of these was a left lingular lobe infiltrate detected, which cleared within 2 weeks with antibiotics. Minimal fibrotic changes were observed on chest X-ray. In August 1975, the patient's respiratory difficulties worsened, and increased fibrosis was evident on chest X-ray at this time, but no acute infiltrates were identified. Pulmonary function studies were consistent with severe restrictive pulmonary disease. Forced vital capacity and forced expiratory volume were measured at 14% of the predicted values. Barium swallow revealed a normal esophagus, with no evidence of regurgitation. The respiratory difficulty became progressively worse, and in July 1976, on room air, PO_2 was 33 mm Hg; PCO_2, 67 mm Hg; and pH, 7.34. Chest X-ray revealed progressive fibrotic changes and a minimal right pleural effusion. No fever or other constitutional symptoms were present at this time. No evidence of brain tumor was seen on CT scan of the head. The patient's condition continued to worsen and he died within 1 week because of respiratory failure.

Autopsy Findings

An autopsy of the brain revealed a residual low-grade astrocytoma involving the pons and medulla, with men-

FIGURE 1.—Extensive fibrosis present subpleurally (hematoxylin and eosin stain, × 116).

ingeal spread around the cerebellum and upper cervical cord. The immediate cause of death, however, appeared to be respiratory failure. At autopsy, the right lung weighed 300 g and the left, 200 g. The surface of the right lung was covered by dense adhesions, while the left pleural surface was smooth. In both lungs, there was prominent subpleural fibrosis, most striking in the right lower lobe. The cut surface of the lungs was firmer than usual, and smaller airways showed mucous plugging. Microscopically, progressive pulmonary fibrosis was present, with lesions of different ages. In large areas, alveolar walls were thickened by fibrosis, were lined by hyperplastic and dysplastic pneumocytes, and showed septal infiltrates of a small number of mononuclear cells and an intra-alveolar accumulation of foamy macrophages, some of which contained a brown pigment. Hyaline membranes lined respiratory bronchioles and alveolar ducts in these regions. In other areas, only focal fibrotic scars were evident, obliterating bronchi and bronchioles. The pleura was thickened, and there was prominent fibrosis in the subpleural regions and in interlobular septa (fig 1). A few bronchi contained mucous plugs. In one area there was a superimposed acute bronchopneumonia. Post-mortem cultures were all negative.

DISCUSSION

The changes in the lungs resemble those seen in other examples of cytotoxic drug-induced pulmonary disease, which produce interstitial pneumonia and fibrosis. The onset in this patient was insidious, and the respiratory problems were progressive. The maximum damage occurred 2 years after discontinuation of therapy. This case emphasizes the necessity of close monitoring for lung damage from cytotoxic drugs.

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Tamoxifen Before and After Orchiectomy in Advanced Male Breast Cancer

Male breast cancer is a rare disease, accounting for approximately 1% of all cases of breast cancer (1). Orchiectomy is the treatment of choice for advanced male breast cancer (2). However, it is not accepted by many patients and other modalities of hormonal treatment must be considered in these cases. The role of additive hormonal therapy has not yet been well-defined in male breast cancer. Tamoxifen, with an overall response rate of 48% in the 32 male patients reported in a review by Patterson et al (3), seems to be an effective and well-tolerated treatment. Moreover, tamoxifen seems to be more effective if employed before orchiectomy. Of nine patients with advanced male breast cancer treated with tamoxifen before orchiectomy (4–9), seven responded (4,5,7–9). Since advanced male breast cancer is too rare to be the subject of controlled studies, further reports are needed to evaluate the efficacy of tamoxifen, especially as primary treatment before orchiectomy (4). We report on four male patients with advanced breast cancer treated with tamoxifen either before or after orchiectomy. The assessment of treatment response was made according to modified International Union Against Cancer criteria (10).

CASE REPORTS

Patient 1

A 64-year-old man was first seen in August 1976 with lung and skin metastases from breast cancer (mastectomy performed in October 1970). In September 1976,

orchiectomy was performed and resulted in partial remission. However, in July 1977 lung and skin metastases progressed and tamoxifen was given at a dose of 20 mg twice a day. In November 1977, skin metastases were nearly undetectable, whereas only a slight decrease of lung metastases was noted. Bone involvement became manifest in December 1977 and chemotherapy was added to tamoxifen, with progression of lung and bone metastases but with complete resolution of skin metastases. The patient died in January 1979 as a result of disseminated disease without evidence of skin lesions.

Patient 2

A 44-year-old man was first seen in December 1975 after mastectomy had been performed 1 month earlier for $T_2,N+,M_0$ breast cancer. Orchiectomy was performed as adjuvant therapy. In April 1980, bone metastases were manifested and tamoxifen was given at a dose of 20 mg twice a day, with subjective improvement until March 1981 when bone metastases progressed. The patient was then treated with chemotherapy and radiotherapy and is presently alive and continuing to receive treatment.

Patient 3

A 70-year-old man was first seen in June 1980 with a cutaneous relapse at the site of the mastectomy performed for breast cancer in September 1978. Despite the administration of tamoxifen at a dose of 20 mg twice a day, progressive disease ensued. Orchiectomy performed in August 1980 induced a complete remission lasting until January 1981. The patient is alive and receiving chemotherapy and radiotherapy.

Patient 4

A 57-year-old man was first seen in January 1981 for inoperable locally advanced breast carcinoma with axillary lymph node involvement. Tamoxifen at a dose of 20 mg twice a day was given, and there was disease progression. Orchiectomy that was performed in March 1981 induced a partial remission. Chemotherapy was then employed and the patient is still alive with his disease under control.

Tamoxifen as primary treatment was ineffective in patients 3 and 4. Subsequent orchiectomy induced objective response in these patients. On the other hand, tamoxifen was effective in patient 1, who was no longer responding to orchiectomy. This finding was also reported in the review of Patterson et al (3). Whether tamoxifen and orchiectomy are non-cross-resistant treatments in advanced male breast cancer remains to be determined in more patients and in association with estrogen-receptor determinations.

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Nasal Cartilage Necrosis Following High-Dose 5-FU: A Case Report

We present a case in which necrosis of the nasal cartilage followed the use of high-dose 5-FU. As far as we know this association has not been previously reported.

In 1971, the patient, a 51-year-old woman, had a total colectomy and ileostomy for ulcerative colitis of 20 years' standing. In April 1973, an adenocarcinoma was excised from the perineum. In February 1974, her pelvis and perineum were treated with radiotherapy (5000 rads in 5 weeks, 200-rad daily fractions) for a local recurrence. She then received chemotherapy with 5-FU (500 mg iv weekly for 1 year). Following further local recurrence in April 1975 she was treated initially with iv vincristine, doxorubicin, 5-FU, methotrexate, and cyclophosphamide (five courses) and then oral cyclophosphamide, methotrexate, and razoxane until July 1978. Chemotherapy was then stopped because the woman was free of disease.

In December 1978, the patient developed further perineal recurrence confirmed histologically, and a computerized tomographic scan showed an associated pelvic mass. At the time, we were aware that the modulation of 5-FU toxicity by allopurinol had been investigated in a dose-escalation study. Stomatitis had become a dose-limiting factor at a dose of 2.25 g/m^2/day, infused over 24 hours for 5 days (1). In addition, it was then thought that allopurinol modulation might improve the therapeutic ratio. For this reason, and following a further 1600 rads to the pelvis, she was given 2.8 g (2 g/m^2/day) of 5-FU as an infusion for 5 consecutive days. Allopurinol (300 mg three times a day) was given concurrently and for 48 hours before the 5-FU. The day after the course was completed the patient noticed stuffiness of the nose associated with gradual swelling. Following this she developed pancytopenia and severe oral mucositis necessitating iv feeding. As her oral symptoms improved, the patient noticed what she thought to be increasing prominence of the dorsal bony nasal hump. On examination, however, it became evident that this was due to gradually increasing saddling of the cartilaginous dorsum, associated with lack of nasal septal cartilage support. Anterior rhinoscopy showed that the mucous membranes of the septum were intact but that the cartilage had absorbed as would be the case following an extensive submucous resection. There was no past history, however, of any nasal surgery or trauma. X-rays of the sinuses and the nasal bones revealed no evidence of residual cartilage, and biopsy of the mucous membranes might have led to a troublesome septal perforation.

The patient was then treated with 5-FU in conventional doses, but after achieving a partial response for 1 year, she died following disease progression.

DISCUSSION

There is no doubt clinically that this patient developed necrosis of her nasal septal cartilage. Known causes of nasal cartilage necrosis such as trauma, syphilis, or cocaine abuse have been excluded. The fact that the event was immediately preceded by high-dose 5-FU suggests a causative link between the two. There have been no previous reports of nasal septal cartilage necrosis with either 5-FU or allopurinol, and neither drug is concentrated in cartilage (2). It is possible that the patient developed nasal mucosal edema as part of her generalized mucositis and that this led to compression of the perichondrium and to avascular necrosis.

We have now treated a total of 34 patients with high-dose 5-FU and allopurinol modulation, using the lower dose of 5-FU (1.5 g/m^2), infused over 6 hrs. This is the subject of a separate report (3). We have had no other cases of nasal cartilage necrosis, although there were three cases of pancytopenia and three cases of mucositis. Unfortunately, there was no clinical benefit.

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Adverse Reactions to AMSA in Medical Personnel

We have observed unusual reactions to AMSA in nine pharmacy and nursing personnel who have been involved in mixing the drug in preparation for administration.

A 21-year-old pharmacy technician developed an urticarial rash on the arms and trunk within minutes after mixing AMSA in a horizontal vented hood within the hospital pharmacy. He was given 50 mg of diphenhydramine hydrochloride orally and the rash subsided within 4 hours. Two days later, although he wore surgical gloves, the same reaction occurred following the preparation of the drug. When he prepared the drug 5 days later, wearing gloves, surgical mask, and surgical gown, the reaction developed for a third time. No other symptoms were noted. The pharmacist, a 30-year-old white female, relieved the technician of this responsibility and prepared the drug solution herself 2 days later. While adding the diluent, the vial shattered and some of the drug spilled on her hands and clothing. She immediately washed the drug from her hands but did not change clothes. Four hours later she developed nausea followed by vomiting. Except for light-headedness, she had no other symptoms. The nausea and vomiting abated after 4 hours and she felt well the next morning.

Several weeks earlier, six of eight pharmacy technicians and nurses responsible for the handling of AMSA in another hospital had experienced unusual symptoms within days of each other. In each instance the symptoms developed after handling AMSA. The symptoms began between 5 minutes and 3 hours after mixing the drug and included nausea, light-headedness, headache, sharp epigastric pain, and malaise. The symptoms in this group of persons lasted from 1 to 12 hours. One technician subsequently developed similar symptoms while standing adjacent to the area where the drug was being mixed but did not develop symptoms when he mixed the drug a few days later using a vertical vented hood.

A few weeks later, a pharmacy technician at a third hospital developed similar symptoms of nausea, light-headedness, malaise, and headache after mixing AMSA.

These apparent drug reactions prompted the recommendation that a vertical vented hood be used for mixing of the drug. Horizontal vented hoods were not recommended because the direction of airflow would result in potential direct drug exposure as the drug is being prepared. No additional reactions have been reported in pharmacy or nursing personnel after 3 months of observation following the recommendation that a vertical hood be used.

In summary, nine pharmacy and nursing personnel have developed symptoms compatible to the toxic effects of AMSA. Seven of the personnel have developed nausea, light-headedness, headache, and malaise within hours after mixing the drug. One person developed nausea and one developed an urticarial rash on three separate occasions after mixing AMSA. Additional reactions appear to have been prevented by mixing the drug in a vertical vented hood in the pharmacy.

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ANNOUNCEMENTS

Fifth Annual San Antonio Breast Cancer Symposium

San Antonio, Texas, November 5–6, 1982

The program of scientific presentations will be complemented by state of the art lectures by invited speakers: Anthony B. Miller, National Cancer Institute of Canada, "Screening Breast Cancer"; William L. McGuire, The University of Texas Health Science Center at San Antonio, "New Approaches to Understanding the Biological Behavior of Breast Cancer"; Marvin Zelen, Harvard University School of Public Health, "The Innovative Approaches to the Design of Clinical Trials in Breast Cancer"; and James H. Goldie, Cancer Control Agency of British Columbia, "Chemotherapeutic Strategy Based Upon Mutation to Resistance in Breast Cancer."

Symposium coordinators are Drs. William L. McGuire and Charles A. Coltman, Jr.

For further information, contact: Terri McDaniel, RN, Cancer Therapy and Research Center, 4450 Medical Dr, San Antonio, TX 78229 (telephone: 512-690-0655).

Second Winter Symposium on Hematologic Malignancies

Snowbird, Utah, February 12–19, 1983

This symposium will feature a multidisciplinary faculty from the University of Arizona Cancer Center. They will review recent diagnostic and therapeutic advances in the lymphomas, leukemias, and multiple myeloma.

For further information, contact: Stephen E. Jones, MD, Section of Hematology and Medical Oncology, University of Arizona Cancer Center, Tucson, AZ 85724.

Treatment of Advanced Gastrointestinal Cancer

Padova, Italy, June 23–24, 1983

The aim of this symposium, sponsored by the European Organization for Research on Treatment of Cancer, is to evaluate the current modalities available for the treatment of advanced tumors of the gastrointestinal tract. The meeting will focus on chemotherapy, radiotherapy, and surgery, either alone or in combination. The program will include invited presentations and free communications. Free communications will be selected on the basis of submitted abstracts. Abstracts should be received by March 31, 1983, and addressed to Pr. M. Lise, Director, Istituto di Patologia Chirurgica I°, Universita degli Studi di Padova, 64 via Gattamelata, 35100 Padova, Italy.

Seating is limited and preregistration is recommended. The registration fee is $90.

For further information, contact: Ms. D. Eeckhoudt, Executive Secretary, EORTC Data Center, 1 rue Héger-Bordet, 1000 Brussels, Belgium (telephone: (2) 539.30.20 - Telex: 22773).

Two International Conferences on Cancer Treatment

Zürich, Switzerland, July 18–23, 1983

The Division of Oncology at the Universitätsspital Zürich is hosting two international conferences titled "Present Status of Interferon in Clinical Cancer Research" and "Recent Developments of In Vitro Tests for Predicting Response to Anti-Cancer Treatment." Due to the current importance and recent progress in these two subjects, a large number of active participants are expected from around the world.

Among the 20 guest speakers that will take part are: Drs. Lindenmann and Weissmann (Zürich); Salmon and Durie (USA); Steel and Courtenay (UK); and Silvestrini (Italy). The program for these meetings will be ready by September 1982.

For further information, contact: Dr. V. Hofmann, Universitätsspital Zürich, Department of Internal Medicine, Division of Oncology, 8091 Zürich, Rämistrasse 100, (telephone 01 2552214).

Clinical Trials in Cancer Medicine: Past Achievements and Future Prospects

Venice, Italy, October 19–21, 1983

The sixth International Bristol-Myers Symposium will be organized by Drs. Umberto Veronesi and Gianni Bonadonna of the Istituto Nazionale Tumori, Milan, Italy, and will take place at the Fondazione Cini. The meeting will be directed to surgical, medical, and pediatric oncologists, radiation therapists, and medical statisticians. The format of the symposium will include a series of presentations followed by discussion. Known cancer specialists from the United States and Europe will critically review the design and the results of major therapeutic trials performed during the past two decades in various neoplastic diseases of adults and children. All formal presentations will be collected in a book edited by U. Veronesi and G. Bonadonna, and will be published in 1984 by Academic Press, New York.

English will be the official language at the symposium and no simultaneous translation will be provided. The registration fee is $150 and lunch will be provided at the Fondazione Cini on October 20 and 21. Only the first 550 applicants who return their registration and hotel forms and pay the fee before March 31, 1983, will be accepted.

For further information, contact: Mr. Renzo Polacco, Trident, Gran Viale 2, Lido di Venezia 30126, Italy (telephone: 041-767.188 or 760.555 - Telex: 410120), before December 31, 1982.

New Drugs in Cancer Therapy

Brussels, Belgium, December 15–17, 1983

The Fourth European Organization for Research on Treatment of Cancer-National Cancer Institute (EORTC-NCI) Symposium will focus on phase I and phase II clinical data including drug pharmacology with investigational anticancer agents. Preclinical aspects in the new drug development area will also be covered, with particular emphasis on the role of the human stem cell assay in the screening of new antitumor drugs. The program will include invited presentations and free communications. Free communications will be selected on the basis of submitted abstracts. Abstracts must be received at the EORTC Data Center by October 31, 1983. Seating is limited and preregistration is recommended. The registration fee is $135.

For further information, contact: Dr. M. Rozencweig or Dr. M. Staquet, EORTC Data Center, 1 rue Héger-Bordet, 1000 Brussels, Belgium (telephone: (2) 539.30.20., telex: 22773).

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